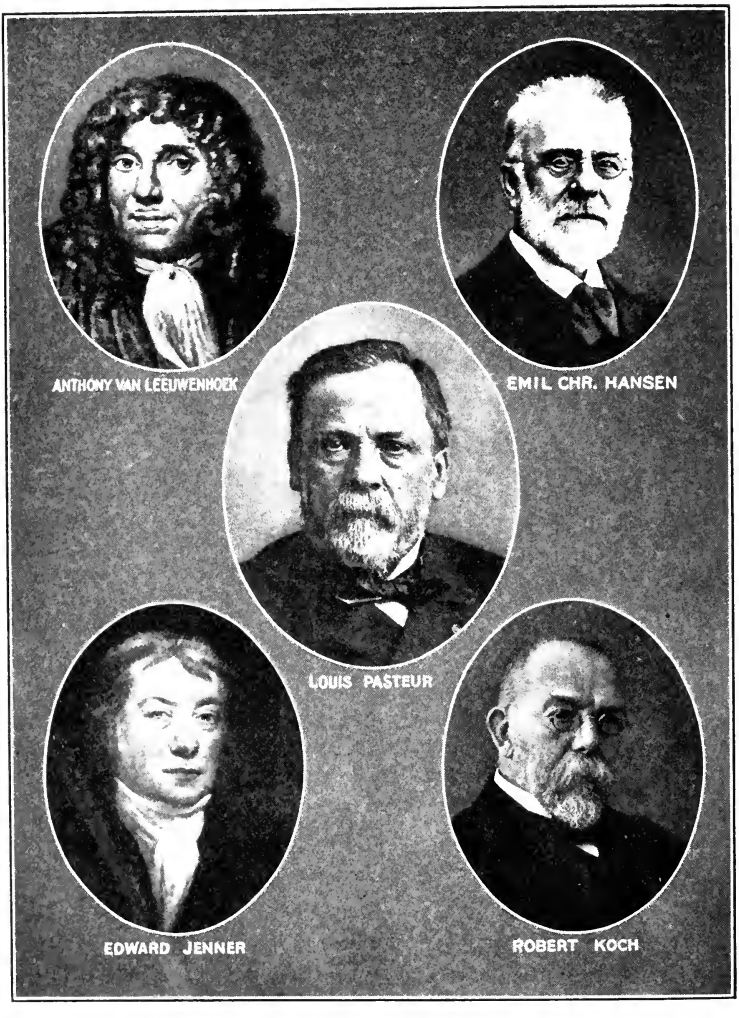
MICROBIOLOGY

MARSHALL


ANTHONY VAN LEEUWENHOEK
EMIL CHR. HANSEN
LOUIS PASTEUR
EDWARD JENNER
ROBERT KOCH

MICROBIOLOGY

A TEXT-BOOK OF

MICROÖRGANISMS GENERAL AND APPLIED

CONTRIBUTORS

F. T. Bioletti, *Berkeley, California.*
R. E. Buchanan, *Ames, Iowa.*
M. Dorset, *Washington, D. C.*
S. F. Edwards, *Lansing, Michigan.*
E. Fidlar, *London, Ontario.*
W. D. Frost, *Madison, Wisconsin.*
A. Guilliermond, *Lyons, France.*
N. MacL. Harris, *Chicago, Illinois.*
F. C. Harrison, *Macdonald College, Que., Canada.*
E. G. Hastings, *Madison, Wisconsin.*
H. W. Hill, *London, Ontario.*
W. E. King, *Detroit, Michigan.*
J. G. Lipman, *New Brunswick, New Jersey.*
W. J. MacNeal, *New York, New York.*
E. F. McCampbell, *Columbus, Ohio.*
Z. Northrup, *East Lansing, Michigan.*
E. B. Phelps, *Washington, D. C.*
O. Rahn, *Elbing, Germany.*
L. F. Rettger, *New Haven, Connecticut.*
M. H. Reynolds, *University Farm, St. Paul, Minnesota.*
W. G. Sackett, *Fort Collins, Colorado.*
W. A. Stocking, *Ithaca, New York.*
C. Thom, *Washington, D.C.*
J. L. Todd, *Montreal, Quebec.*
E. E. Tyzzer, *Boston, Massachusetts.*

EDITED BY

CHARLES E. MARSHALL

Amherst, Massachusetts

PROFESSOR OF MICROBIOLOGY AND DIRECTOR OF GRADUATE SCHOOL
MASSACHUSETTS AGRICULTURAL COLLEGE

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CONTRIBUTORS

BIOLETTI, FREDERIC T., M. S.
Professor of Viticulture and Enology, Viticulturist of Experiment Station, University of California, Berkeley.

BUCHANAN, R. E., B. S., M. S., PH. D.
Professor of Bacteriology, Bacteriologist of Experiment Station, and Dean of Industrial Science, Iowa State College, Ames.

DORSET, M., B. S., M. D.
Chief of Biochemic Division, U. S. Bureau of Animal Industry, Washington, D. C.

EDWARDS, S. F., B. S., M. S.
Formerly Professor of Bacteriology, Ontario Agricultural College, Guelph, Canada. Director of The Edwards Laboratories, Lansing, Michigan.

FIDLAR, EDWARD, B. A., M. B.
Chief of Division of Pathology; Pathologist of London Asylum and of Victoria Hospital; Professor of Pathology, W. U. Medical Faculty; Bacteriologist of London Board of Health, London, Ontario.

FROST, W. D., PH. D., D. P. H.
Professor of Agricultural Bacteriology, University of Wisconsin, Madison.

GUILLIERMOND, A. DOCTEUR ÈS SCIENCES.
Professor of Botany, University of Lyon, France.

HARRIS, N. MACL., M. B.
Assistant Professor of Bacteriology, University of Chicago, Chicago.

HARRISON, F. C., D. Sc., F. R. S. C.
Principal and Professor Bacteriology, Macdonald College (Faculty of Agriculture, McGill University), Macdonald College, Que., Canada.

HASTINGS, E. G., M. S.
Professor of Agricultural Bacteriology, Bacteriologist of Experiment Station, University of Wisconsin, Madison.

HILL, H. W., M. B., M. D., D. P. H.
Director of Institute of Public Health; Chief of Division of Epidemiology; Professor of Public Health, W. U. Medical Faculty; Medical Officer of Health of London, London, Ontario.

KING, WALTER E., M. A., M. D.
Formerly Professor of Bacteriology and Bacteriologist of Experiment Station, Kansas Agricultural College, Manhattan. Assistant Director of Research Laboratory, Parke, Davis & Co., Detroit, Michigan.

LIPMAN, JACOB G., PH. D.
Dean of Agriculture, Rutgers College; Director of Experiment Station, New Brunswick, New Jersey.

MACNEAL, WARD J., PH. D., M. D.
Professor of Bacteriology and Director of the Laboratories, New York Post-Graduate Medical School and Hospital, New York.

MCCAMPBELL, EUGENE F., PH. D., M. D.
Professor of Preventive Medicine, Dean of the Medical College, Ohio State University.

NORTHRUP, Z., M. S.
Assistant Professor of Bacteriology and Hygiene, Michigan Agricultural College; Assistant Bacteriologist and Hygienist of Experiment Station.

PHELPS, EARLE B., B. S.
Professor of Chemistry, Hygienic Laboratory, U. S. Public Health Service, Washington, D. C.

RAHN, OTTO, PH. D.
Formerly Assistant Professor of Bacteriology, Illinois University, Urbana. Now Elbing, Germany.

RETTGER, L. F., PH. D.
Assistant Professor of Bacteriology and Hygiene (in Sheffield Scientific School), Yale University, New Haven, Connecticut.

REYNOLDS, M. H., B. S., M. D., D. V. M.
Professor of Veterinary Medicine and Surgery, Agricultural College, University of Minnesota; Chairman, Veterinary Division, Experiment Station, University Farm, St. Paul.

SACKETT, WALTER G., B. S.
Bacteriologist, Colorado Experiment Station, Colorado Agricultural College, Fort Collins.

STGCKING, W. A., M. S. A.
Professor of Dairy Industry, Cornell University, Ithaca, New York; Dairy Bacteriologist of the Experiment Station.

THOM, CHARLES, PH. D.
Mycologist, Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.

TODD, J. L., B. A., M. D., D. Sc.
Associate Professor of Parasitology, McGill University, Montreal.

TYZZER, E. E., A. M., M. D.
George Fabyan Professor of Comparative Pathology; formerly Director of the Cancer Commission of Harvard University, Boston, Massachusetts.

INTRODUCTION TO THE SECOND EDITION

The continued and growing demand for "*Microbiology*" has caused the contributors to undertake a thorough revision. In this they have been guided by the recent developments in this branch of science, and also by a desire to adjust and rearrange in the light of constructive suggestions and criticisms.

The primary purpose of this text-book is to place in the hands of college students an elementary technical treatise of the subject matter included. No effort has been made to review or cite literature, for to do either would expand the volume beyond useful limits. To provide an introductory text-book mainly for recitations, or for a supplement to lecture or laboratory courses, is about all that can be satisfactorily comprehended in a single project.

The cytological aspect of microbiology has seemed to us to deserve some emphasis, for it has become quite definite and has been suggestively indicating much of real value in connection with the active life processes of the cell and microbic activities in agriculture, medicine and wherever microbiology is applicable.

The significance of "Intestinal Microbiology" has required a short chapter for its proper presentation.

It has also been found desirable to treat the microbial diseases of insects, a growing subject, in a distinct chapter.

The study of microörganisms flounders in a fog of unsettled ideas for a proper designation. Whether it should be called Protistology, Microbiology, Bacteriology, Mycology, or something else must be left for the future to determine.

CHARLES E. MARSHALL, EDITOR.

AMHERST, MASSACHUSETTS.

INTRODUCTION TO THE FIRST EDITION

By a process of adaptation and growth, the branch of science commonly recognized as "*Bacteriology*" has for many years included, besides the bacterial forms, those microörganisms yielding to the same laboratory methods of study and investigation. This is a policy or purpose instituted by Pasteur. It is also the result of investigations and added knowledge, more definite arrangements of available facts, and the highly specialized training required for the work. In short, technic together with the economic relations of the subject-matter has no little influence in placing limitations. In the light of such circumstances, it appears more pertinent to designate this text-book as "*Microbiology*," perhaps not the best term, but one much in accord with French usage.

Agriculture, Domestic Science and certain other courses in scientific schools and colleges call for the treatment of the subject in such a manner as to make it basic to the interpretation of such subjects as air impurities, water supplies, sewage disposal, soils, dairying, fermentation industries, food preservation and decomposition, manufacture of biological products, transmission of disease, susceptibility and immunity, sanitation, and control of infectious or contagious diseases. A strong effort has been made to provide the fundamental and guiding principles of the subject and to show just how these principles fit into the subjects of a more or less strictly professional or practical nature. Here the instructional work of the microbiologist stops in most educational institutions and the instruction of the practical or professional man begins.

Because of the extreme massiveness and diversity of the subjects, Agriculture and Domestic Science and Industrial Vocations in general, a comprehensive consideration of the subject is demanded. Elimination of many features not only becomes difficult but really precarious, because so many avenues are open to the student that pertinency cannot

always be foreseen or determined. It is well to remember, too, that such aggregate subjects as Agriculture and Domestic Science, unlike Engineering and Medicine, because of their youth, have not developed to that stage in their educational history where practice and the science upon which practice should be founded are amalgamated. The practical man in Agriculture, and Applied Sciences generally, too frequently is so extremely traditional in his practice that he utterly fails to separate the true from the false, or, in other words, does not exercise his discriminative powers at all, but depends entirely upon so-called haphazard methods and self-willed processes. This factor operates against the proper development and logical study of any branch of science in its relation to the farmer, or manufacturer.

The plan of a text-book in Microbiology which seeks to furnish basic principles, to train the mind in logical development and adjustment, and to prepare the student to undertake an intelligent study of strictly professional or practical subjects, must assume a definite and systematic arrangement. With this in mind, the text has been divided into three distinct parts: *Morphological and Cultural*, or that which deals with forms and methods of handling; *Physiological*, or that which deals strictly with functions, the key to the applied; *Applied*, or that which reaches into the application of the facts developed to the problems met in the study of professional or practical affairs.

In a text-book, *the product of several hands*, there is the most serious difficulty in obtaining unity of thought and expression without repetition; besides, that very conspicuous weakness of emphasizing some features unduly while other features of importance are scarcely mentioned, confronts us. A most earnest attempt has been made to overcome these faults as far as possible, but a complete mastery of them cannot be expected in the first product, However, what is lacked in unity and continuity of expression and in balance we sincerely hope will be made up, in part at least, by the selection and the value of the material contributed.

Laboratory features of microbiology have been eliminated wherever it has been practicable. Should any demonstration be added or needed, we have felt that they may be easily supplied by the instructor, who, of course, will be governed by local facilities and conditions. Although no space has been given to laboratory exercises, is should not be gathered that the authors of this book are any the less earnest in

urging a well-organized laboratory course to supplement the general instruction as an essential factor to a working appreciation of the subject.

In matters of spelling, new words, and phrases, conservatism has controlled. Abritrary decisions and selections have been forced in several instances to secure clearness, consistency and definiteness. It is painfully evident to anyone attempting to bring system out of the confusion and chaos existing in many fields of microbiological action that some rearrangement ought to be undertaken. As usual, however, this will be very slow on account of the many almost insurmountable difficulties.

We need and invite helpful suggestions and criticisms at all times, for a valuable text-book of the nature of this is one of slow growth and development and not of "sport evolution." The editor is certain that each contributor will welcome suggestions and, further, will be in far better position to judge his own contribution after the material appears in book form and has been submitted to students for which it is designed.

No one better than the editor realizes fully the sympathetic part played by the contributors. If any merit attaches to this book as it finds its place in microbiological instruction, *such merit* should be recognized as due the contributors whose unselfish aims have made it possible.

CHARLES E. MARSHALL, EDITOR.

AMHERST, MASSACHUSETTS.

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HISTORY OF MICROBIOLOGY*

Geronimo Fracastorio, of Verona, was born in 1484, studied medicine in Padua, and published a work in Venice in 1546, which contained the first statement of the true nature of contagion, infection, or disease organisms, and of the modes of transmission of infectious disease. He divided diseases into those which infect by immediate contact, through intermediate agents, and at a distance through the air. Organisms which cause disease, called *Seminaria contagionum*, he supposed to be of the nature of viscous or glutinous matter, similar to the colloidal states of substances described by modern physical chemists. These particles, too small to be seen, were capable of reproduction in appropriate media, and became pathogenic through the action of animal heat. Thus Fracastorius, in the middle of the sixteenth century, gave us an outline of morbid processes in terms of microbiology.

Athanasius Kircher, in 1659, demonstrated the presence of "minute living worms in putrid meat, milk, vinegar, etc.;" but he did not describe their form and character, and it is doubtful whether he ever saw microörganisms.

In the year 1683 Antonius van Leeuwenhoek, a Dutch naturalist and a maker of lenses, communicated to the English Royal Society the results of observations which he had made with a simple microscope of his own construction, magnifying from 100 to 150 times. He found in water, saliva, dental tartar, etc., what he termed "animalcula." He described what he saw, and by his drawings showed both rod-like and spiral forms, both of which, he said, had motility. In all probability, the two species he saw were those now recognized as *Bacillus buccalis maximus* and *Spirillum sputigenum*. Leeuwenhoek's observations were purely objective and in striking contrast with the speculative views of M. A. Plenciz, a Viennese physician, who in 1762 published a germ theory of infectious diseases. Plenciz maintained that there was a special organism by which each infectious disease was produced,

*Prepared by F. C. Harrison.

that microörganisms were capable of reproduction outside of the body, and that they might be conveyed from place to place by the air.

The important rôle that the compound microscope has played in microbiology calls for something regarding the invention of this instrument—an invention which antedates Leeuwenhoek's discovery by nearly 100 years.

The first compound microscope was made by Hans Jansen and his son Zaccharias, in 1590, at Middelburg, in Holland. The instrument was composed of two lenses mounted in tubes of iron; a representation of it, made from the original and still kept at Middelburg, is shown in Fig. 1. From that date the microscope gradually improved. In 1844 the immersion lens was introduced by Dolland. In 1870 Abbé brought out the substage condenser, which still bears his name. Apochromatic lenses and many minor improvements were introduced by the firm of Zeiss about 1880.

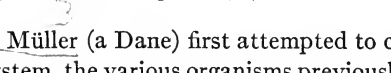


FIG. 1.—Longitudinal section of a compound microscope made by Zacharias Jansen (1590). *a*, Microscope tube; *b*, objective tube; *c*, ocular.

In 1786 O. F. Müller (a Dane) first attempted to classify, according to the Linnean system, the various organisms previously discovered, and characterized four or five genera—among them, the genus *Vibrio*, in which, under the terms *bacillus*, *lineola*, and *spirillum*, we recognize forms that correspond with our "*bacteria*."

From the middle of the eighteenth century until well on into the nineteenth, the history of bacteriology is largely the story of a controversy between those who believed that minute living organisms, such as those above referred to, were produced from inanimate substances, and that their formation was *spontaneous*. Philosophers, poets, and common people of the most enlightened nations accepted this doctrine down to the eighteenth century. The hypothesis regarding this formation was known as that of "spontaneous generation," "heterogenesis," and "abiogenesis." The opponents of this theory denied the possibility of a transition from a lifeless to a living condition, and contended that all life came from preëxisting life—a theory aphoristically summed up in the phrase "*omne vivum ex vivo*." Such was the doctrine of Biogenesis—life only from life.

In 1668, Francisco Redi, an Italian, distinguished alike as scholar, poet, physician, and naturalist, expressed the idea that life in matter is always produced through the agency of preëxisting living matter; but the beginnings of the real controversy date from the publication of Needham's experiments in 1745. The English divine boiled some meat extract in a flask, made the flask air-tight, and left it for some days. When the flask was opened, he found in it what he termed "infusoria." He naturally concluded that all life had been killed by boiling; and, as the entrance of fresh life from the outside was prevented by the closing of the flask, he considered that the living infusoria must have originated spontaneously from the inanimate constituents of the broth.

Twenty years later Abbé Spallanzani alleged that the development of the infusoria "in an infusion maintained at boiling-point for three-quarters of an hour was possible only, provided air, which had not been previously exposed to the influence of fire, had been admitted." Objections were made to these experiments and the controversy went merrily on. Gradually experimental evidence accumulated—resulting largely from the work of Franz Schulze, and the discovery by Schroeder and Dusch in 1853, that putrescible fluids will not decay after boiling, if protected from the bacteria of the air by means of a cotton-wool filter or plug; and the epoch-making experiments of Pasteur in 1860, with the now well-known Pasteur flask, showed conclusively that the hypothesis of spontaneous generation, or abiogenesis, could not be proved.

Liebig, the celebrated German chemist, strenuously opposed the theories of Pasteur; his authority and the brilliancy of his expositions influenced the scientific world during the period 1840–60. To Liebig, fermentation was a purely chemical phenomenon unassociated with any vital process; and he treated Pasteur's results with disdain. "Those who pretend to explain the putrefaction of animal substance by the presence of microörganisms," he wrote, "reason very much like a child who would explain the rapidity of the Rhine by attributing it to the violent motions imparted to it in the direction of Bingen by the numerous wheels of the mills of Mayence." Again and again Liebig formally denied the correctness of Pasteur's assertions; finally Pasteur challenged him to appear before the Academic Commission to which they would submit their respective results. Liebig, however, did not accept the challenge; the victory was with the French savant.

In 1841 Fuchs investigated some blue and yellow milk. He examined it with the microscope and discovered the presence of organisms. He succeeded in cultivating the "blue milk" microbe in mallow slime, and re-developed the blue color in milk by introducing some of his culture. The organisms obtained were sent to Ehrenberg, who named them *Bacterium syncyaneum*, now known as *B. cyanogenus*, *Ps. syncyanea* and *B. synxanthus*, a name which is still retained in the literature.

Since 1860 the master mind of Louis Pasteur has dominated the realm of microbiology. His epoch-making discoveries were largely due to his intuitive vision, his skill in device and in the adaptation of means to ends, his prodigious industry, and the enthusiasm and love with which he inspired his associates. Trained as a chemist, his first appointment was to a professorship of chemistry, and his earliest research dealt with problems in molecular chemistry and physics. On his being elected Dean of the Faculty of Sciences at Lille, he commenced to study fermentation. His work in this field was soon followed by important results: the discovery of the organisms which produce lactic and butyric fermentation, and of anaerobic life, or life which flourishes without free oxygen. He devised an improved method of making vinegar, and demonstrated the presence of the *acetic* organism which he named *Mycoderma aceti*. Later he studied the diseases of wine, and discovered that bitterness or greasiness was due to a special ferment, and suggested the heating of wines in closed bottles to a temperature of 60°, in order to kill the injurious microörganisms. This process, since called pasteurization, is now largely used, and makes it possible for manufacturers and merchants to keep and export wine without losing its flavor or bouquet. It is interesting in this connection to note that a French confectioner named Appert published, in 1811, his method of preserving fruits, vegetables, and liquors by heating and sealing, and hence may be looked upon as the founder of the packing and canning industry.

In 1864–65 the silk districts of that region of France, known as the Midi, suffered such serious losses that the yield of cocoons fell from twenty-six million kilograms to four million, which entailed a loss of twenty million dollars and caused widespread distress and poverty. An epidemic had broken out among the silk-worms—the dread disease known as Pébrine. Pasteur was induced to make an in-

vestigation as to the best means of combating the epidemic; and, after several years of study, he found the organism causing the disease, suggested remedies, and brought back wealth to the ruined communities, but at the cost to himself of impaired health and partial paralysis.

Pasteur's results were very suggestive; and one outcome of his work was that between 1870 and 1880 several important discoveries were made by other investigators. Prior to the dates mentioned, the mortality from blood poisoning, gangrene, and other infections following operations was extremely high. Surgeons regarded such a result as inevitable, and many agreed with the saying of Velpeau, that "the prick of a pin is the open door to death;" but, in 1860, Joseph Lister, an Edinburgh surgeon, began to study the possible rôle of microbes in the infection of wounds. By sterilizing his instruments, sponges, ligatures, etc., and using antiseptics, he was able to obtain such a high percentage of recoveries that in two years he saved thirty-four patients out of forty—a percentage unheard of up to that time. Hence the origin of the antiseptic and aseptic methods of surgery is traceable to Lister's efforts. Lister's methods, suggested by the ideas of Pasteur, have rendered possible the marvelous surgery of the present day, banished hospital gangrene, and robbed confinement of its terrors.

To Lister must also be given the honor of devising the first practical way of obtaining a pure culture of bacteria by means of high dilutions. By using this method, Lister obtained some idea of the different fermentations of milk, such as souring, curdling, etc. He also confirmed the conclusion of Robert Hall (1874), that milk could be obtained from the animal in a sterile condition, thus proving that the souring of milk was caused by organisms from some external source.

In 1872, F. Cohn's System of Classification, based on morphological characters, appeared. He distinguished six genera—micrococcus, bacterium, bacillus, vibrio, spirillum, and spirochæte; four years later this investigator made the important discovery of endospores (spores formed within cells), and noticed that organisms in this state were more resistant to heat than the rods from which they were derived. This fact was observed in the well-known "*hay bacillus.*"

In 1871, Weigert succeeded in staining bacteria with picro-carmine; but it was not until 1876 that he used the aniline colors, or dyes, for this purpose, and thus opened up a new field which was exploited with such

beautiful results by Ehrlich, Koch, Gram, and others. The staining of microörganisms rendered it possible to obtain pictures of them by photographic methods; the art of photomicrography developed thus rapidly.

In 1879, Miquel discovered bacteria which grew or developed at temperatures between 65°* and 75°. He isolated them first from the waters of the Seine, and subsequently from dust, manure, and other substances. Later researches have shown that these thermophilic organisms play important rôles in various fermentations.

The ninth decade of the last century was prolific in important bacteriological events. Discovery followed discovery in rapid succession. In 1880, Laveran, a French military surgeon, discovered the protozoön of malaria; in 1881 Robert Koch introduced the poured gelatin and agar plate, which made it possible to obtain pure cultures without difficulty. Investigators were quick to take advantage of this method; and notable results followed. Eberth and Gaffky discovered the bacillus of typhoid fever, and succeeded in growing it in culture media. In 1882, Loeffler and Schütz discovered the bacterium which causes glanders; and in the following year Koch isolated the vibrio of Asiatic cholera from the intestines of cholera patients. In 1883 Klebs described the diphtheria bacterium; and, in 1884, Loeffler grew the organism in pure culture.

In 1884, Koch published his results on the etiology of tuberculosis, in a paper which will remain as a classical masterpiece of bacteriological research, owing to the difficulty of the task and the thoroughness of the work. Not only did Koch show the tubercle bacterium by appropriate staining methods, but he succeeded in obtaining pure cultures of it and in producing tuberculosis by inoculation with his isolated cultures.

In 1885, Nicolaier observed the tetanus bacillus in pus produced by inoculating mice and rabbits with soil; later, in 1889, Kitasato isolated this organism, and showed that the cause of the failure in earlier attempts to isolate it were due to the fact that it could grow only in the absence of free oxygen. The specific infecting agents in pneumonia were discovered by Friedlander and Fraenkel about this time, as were also several organisms associated with inflammation and suppuration, such as the *Streptococcus pyogenes* and the *Staphylococcus pyogenes*, discovered by Rosenbach, and the green pus germ (*Pseudomonas pyocyanea*) by Gessard.

*All temperatures are stated in Centigrade scale, unless otherwise indicated.

While these discoveries were taking place, largely in Germany, Pasteur had been engrossed with his prophylactic studies. In 1880, he discovered a method of vaccination against fowl cholera; and in 1881 he published his method of vaccination against anthrax. On a farm at Pouilly le Fort, sixty sheep were placed at Pasteur's disposal; ten of these received no treatment, and twenty-five were vaccinated. Some days afterward the latter was inoculated with virulent anthrax, and also twenty-five which had received no vaccine. The twenty-five non-vaccinated sheep died, and the twenty-five vaccinated ones remained healthy and in the same state as the ten control animals. This convincing experiment was followed by others; and, in the twenty-five years immediately following the introduction of the method, more than ten million animals were vaccinated in France alone, with excellent results. In 1885, as the result of much animal experimentation, Pasteur related to the Academy of Sciences his discovery of a method of vaccination against rabies, or hydrophobia; and six months after the successful treatment of the first case, 350 persons bitten by rabid dogs were vaccinated. An institute for the preparation of vaccines was built by public subscription and named the Pasteur Institute; and since that date more than thirty similar establishments have been founded in different parts of the world.

This eighth decade, so pregnant with discoveries of the utmost importance to medicine and surgery, was also notable for its discoveries in agricultural bacteriology. The honor of having been the first to work out the causal relation between a specific microbe and a plant disease belongs to Burrill, who discovered the organism of Fire or Pear Blight; and in 1883 to 1888 Wakker discovered the bacillus which produces the "yellows" of the hyacinth, a disease of considerable economic importance in Holland. To Beyerinck, Hellriegel, and Wilfarth we owe our earlier knowledge of the development and morphology of the nitrogen-fixing organism which produces the nodules or tubercles on the roots of legumes. In 1888 Winogradsky isolated from soils nitrifying microbes which grew in a medium devoid of all traces of organic matter. During this period, Hansen's investigations along the line of the fermentation industry were most important. He devised methods for securing pure cultures of yeasts starting from a *single cell*, showed that yeasts produced diseases in beer, and established the method of

identifying yeasts by observing their microscopic appearance, the formation of ascospores, and the production of films.

The tenth decade of the nineteenth century was almost as prolific in discovery as the ninth. In 1890 Behring discovered the antitoxin for diphtheria, as a result of the pioneer work on toxins by Roux and Yersin. Five years later, this serum came into general use as a curative agent; and the efficiency of the treatment is shown by a comparison of the death rate from diphtheria before and after the introduction of the antitoxin. The average annual death rate from diphtheria in eight large cities, during the period 1885–94, was 9.74 per 10,000 of the population before the use of antitoxin; and during the antitoxin period of 1895–1904 it was 4.29.

The subsequent researches on the constitution of toxins and antitoxins by Ehrlich, Metchnikoff, Madsen, and others have been productive of a better understanding of the problems of immunity.

In 1892 Pfeiffer discovered the organism of influenza or grippe; and in 1894 Yersin and Kitasato independently discovered the bacterium of bubonic plague.

The now well-known serum diagnosis of typhoid fever, whereby living and motile typhoid bacilli are clumped and lose their motility when placed in the diluted serum of a patient suffering from the fever, was due to the work of Gruber and Durham, and the exploitation of the method by Widal dates from 1896.

In 1898, Shiga discovered the bacterium of dysentery, and the possible cause of pleuro-pneumonia in cattle was found by Nocard. This latter organism was so minute as to be at the extreme limit of microscopic definition, and suggested that other well-known diseases, such as foot-and-mouth disease, are probably caused by ultra-microscopic organisms.

This year, Ronald Ross worked out the relation between man, the mosquito, and the malarial parasite—a discovery which at once suggested the best means of controlling the disease.

In 1905, Schaudinn definitely established the causal agent of syphilis, a spirochæte-shaped organism, which he named *Treponema pallidum*, and which had escaped earlier discovery on account of its being refractory to the ordinary staining methods.

No one can deny that the progress of microbiology in the last forty years has been extraordinary; but much still remains unknown. The

causes of some diseases have not been discovered. Smallpox, scarlet fever, yellow fever, mumps, whooping-cough, epidemic infantile paralysis, hydrophobia, and others offer an inviting field to the medical microbiologist; and the many problems of soil microbiology call for solution by the agricultural microbiologist. Yet it cannot be said that the laborers are few.

The record of past achievements is an inspiration; and the knowledge that each discovery was the result of persistent and concentrated effort, may give us of the present day firmer faith and greater strength for work in the broad and inviting field before us.

PART I

THE MORPHOLOGY AND CULTURE OF MICRO-ORGANISMS

General*

Microbiology is concerned almost wholly with the field of *unicellular life*. On the one hand, the microbiologist meets the botanist and establishes reciprocal relations with him; on the other hand, he mixes with the zoölogist and delves into studies of mutual interest. Primarily, the *technic* of the microbiologist together with, in part, the *economic bearing* of the subject seems to be the determining factor of limitation.

Assuming, therefore, that the province occupied by microbiologists consists of the study of *unicellular life-forms*, because such limitations have been established by actual studies and investigations, through the instrumentality of microbiological technic, it will be pertinent and clarifying to provide a general graphic outline at the start. By this means the student will be able to locate himself, whether he is just launching or has gotten far out on the troublesome and most fascinating sea of microbiology. The graphic outlines will always be his ready chart.

* Editor.

Outline of Plant Groups*

The following is a diagram of plant groups, showing one scheme of placing the bacteria, yeasts, and molds in relation to other groups. Only a few of the sub-groups can be shown in such a scheme.

- **Plants**
  - Schizophyta (fission-plants)
    - *Schizomycetes (fission-fungi), bacteria.*
    - Schizophyceæ (fission-algæ), blue-green algæ.
  - Thallophyta
    - Algæ
      - Chlorophyceæ—green algæ.
      - Phæophyceæ—brown algæ.
      - Rhodophyceæ—red algæ.
    - Characeæ.
    - Fungi
      - Myxomycetes.
      - Phycomycetes
        - Chytridineæ.
        - Zygomycetes (*Mucors*).
        - Oomycetes
          - Saprolegniaceæ (water fungi).
          - Peronosporaceæ (downy mildews).
      - Ascomycetes
        - Hemiasci (Monascus).
        - Euasci
          - Protoascineæ (*Saccharomyces, Yeasts*).
          - Protodiscineæ.
          - Discomycetes.
          - Plectascineæ (*Aspergillus*).
          - Pyrenomycetineæ.
      - Imperfect Fungi, Conidia only
        - *Penicillium, Fusarium, Alternaria,*
        - *Oidium, Cladosporium, and others.*
      - Basidiomycetes
        - Rusts
        - Smuts
        - Mushrooms.
  - Bryophyta (mosses and liverworts)
  - Pteridophyta (ferns, etc.)
  - Spermatophyta (seed plants).

*Charles Thom.

Outline of Protozoal Groups*

"An Outline Classification of the Protozoa," embracing only parasitic and more especially the pathogenic forms. For discussion of *classification* see p. 130.

- **Protozoa**
  - **Rhizopoda**
    - Entamœba
      - *Entamœba buccalis*
      - *Entamœba coli*
      - *Entamœba tetragena* (*histolytica*)
      - *Entamœba meleagridis*
    - Plasmodiophora
      - *Plasmodiophora brassicæ.*
  - **Flagellata**
    - Leishmania
      - *Leishmania donovani*
      - *Leishmania tropica*
    - Crithidia
      - *Leishmania infantum*
    - Trypanosoma
      - *Trypanosoma gambiense*
      - *Trypanosoma cruzi*
      - *Trypanosoma brucei*
      - *Trypanosoma evansi*
      - *Trypanosoma equinum*
      - *Trypanosoma dimorphon*
      - *Trypanosoma lewisi*
      - *Trypanosoma equiperdum*
    - Trypanoplasma
    - Cercomonas
    - Trichomonas
      - *Trichomonas intestinalis*
      - *Trichomonas vaginalis*
    - Monas
    - Plagiomonas
    - Lamblia
      - *Lamblia intestinalis*
  - **Sporozoa**
    - Gregarina
    - Coccidium
      - *Coccidium cuniculi* (*Eimeria stiedæ*)
      - *Coccidium avium*
    - Hæmosporidia
      - Plasmodium
        - *Plasmodium vivax*
        - *Plasmodium malariæ*
        - *Plasmodium falciparum*
      - Proteosoma
      - Hæmoproteus
      - Lankesterella (and other Hæmogregarines)
      - Hepatozoön
      - Babesia
        - *Babesia bovis* (*bigemina*)
        - *Babesia canis*
    - Sarcosporidia
      - Sarcocystis
        - *Sarcocystis miescheriana*
    - Haplosporidia
      - Rhinosporidium
        - *Rhinosporidium kinealyi*
    - Myxosporidia
      - Myxobolus
        - *Myxobolus pfeifferi*
    - Microsporidia
      - Nosema
        - *Nosema bombycis*
  - **Infusoria**
    - Balantidium
      - *Balantidium coli*

* J. L. Todd. Revised by Ernst E. Tyzzer.

- Parasites of uncertain position
  - Toxoplasma
  - Chlamydozoa
  - Ultramicroscopic viruses
  - Spirochæta
    - *Spirochæta obermeieri*
    - *Spirochæta duttoni*
    - *Spirochæta vincenti*
    - *Spirochæta theileri*
    - *Spirochæta gallinarum*
  - Treponema
    - *Treponema pallidum*
    - *Treponema pertenue*

CHAPTER I*

ELEMENTS OF MICROBIAL CYTOLOGY

Cells and Energids

The microörganisms are confined to cells, such as algæ, molds, bacteria, yeasts, and protozoa, or cytoplasmic masses with a nucleus associated with each (Fig. 2). Some are, however, made up of rows of cells, such as threads of *Cladothrix*, occasionally capable of branching out, like the mycelium of a mold (Fig. 3, *A*). There are also some cells which have a special structure. In each cell are enclosed several nuclei. If certain amœbæ are examined, for example, *Pelomyxa palustris* (Fig. 3, *B*), inside of what appears to be a cell there are found many nuclei. Such cells have not the anatomical value of true cells, but seem to represent as many cells as there are nuclei. Each of these nuclei with the cytoplasm which surrounds it, equivalent to a cell, may be called specifically an *energid*. Some algæ and fungi are made up of threads of cells enclosing several nuclei; each cell included in a thread consequently represents a group of organized elements, the union of several energids in the same anatomical unit (Fig. 3, *A*).

Structure of the Cell

A typical cell is constituted of three essential elements: the nucleus; the cytoplasm; and the cell-membrane.

The general characteristics of these three elements, and, following this, the study of cell reproduction, may now be systematically presented.

The Nuclear Structure.—*General Structure of the Nucleus.*—The nucleus frequently takes in microörganisms the typical form which it assumes in the higher organisms, namely, that of a spherical vesicle limited by a membrane, enclosing a hyaline substance called the *nuclear-fluid*, or *nucleoplasm* (Fig. 21, *A*, *a*, *B*, *a*). In this nuclear

*By A. Guilliermond.

fluid are found: the *nucleolus*, a spherical corpuscle made up of *pyrinin* to which the *chromatin*, a characteristic substance of the nucleus, frequently attaches itself; the *chromatic network*, the thread of which is made up of *linin*, a very slightly chromophilic substance, enclosing some grains, the *grains of chromatin*, which possess a special affinity for basic stains. The chromatin or *nuclein* is the most important substance of the nucleus.

Centriole.—In intimate contact with the exterior of the nucleus and sometimes inside is usually found a small body called the *centrosome*, or, if the dense chromatin alone is considered, the *centriole* (Fig. 20, *B*, *a*). It is a small chromophilic grain which is often surrounded by a clear zone of protoplasm called *archoplasm*.

FIG. 2.

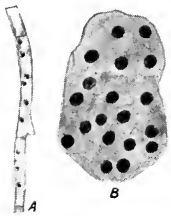


FIG. 3.

FIG. 2.—Cells of *Saccharomyces cerevisiæ*.

FIG. 3.—Cells made up of several energids. *A*, A portion of the mycelium of a mold, *Aspergillus ochraceus*. (*After Dangeard.*) *B*, Cell of an amœba, *Pelomyxa palustris* (*After Doflein*).

Value of the Nucleus.—The nucleus is an organ indispensable to cellular life. It directs for the most part the physiological functions of the cell. It plays an active part in nutrition as is indicated by the fact that the greater part of the products of nutrition or of reserve spreads itself around the nuclear membrane. Finally, it assumes an important rôle in cellular division and in sexual phenomena.

The experiments of Balbiani which have been repeated by other authors show that the cell cannot function without its nucleus. By cutting an infusorial cell in two portions, one of which contains the nucleus and the other only its cytoplasm, Balbiani found that the nucleated part was able to resist the wound which it had received and regenerate the cytoplasm which was lacking; whereas the enucleated portion soon perished.

It does not seem probable, therefore, that cells can exist without their nuclei. Nevertheless, to the present time it has not been possible to find conclusive proof of the presence of a true nucleus in bacteria. The presence in their cells, however, of a great number of small chromatin grains like the chromatin material of nuclei, and their evolution during the formation of spores, force the observer to admit that these represent grains of nuclear substance, and that bacteria have a kind of *diffuse nucleus*, which is scattered in the form of small grains (Fig. 4) in the cytoplasm of the cell.

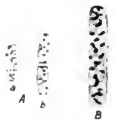


FIG. 4.—Diffuse nuclei of bacteria. *A*, *B. mycoides*. (*After Guillier mond.*) *B*, *Thiothrix tenuis*. (*After Swellengrebel.*)

Forms of Nuclei in Microörganisms.—The nucleus of primitive microörganisms is far simpler than in the higher forms, where it becomes fairly complex. Consequently in the *Cyanophyceæ* or blue algæ, the lowest of all algæ, the nucleus is in a very primitive state. It is large, not separated from the cytoplasm by a membrane, and is made up simply of a nuclear fluid and a chromatic network. The cytoplasm is confined to a thin cortical layer and the nucleus nearly fills the cell (Fig. 5).

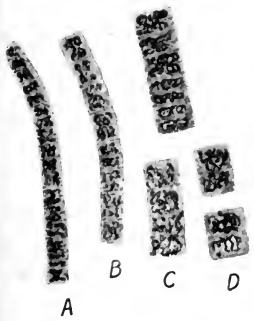


FIG. 5.—Nuclei of *Cyanophyceæ*. *A*, Thread of *Rivularia bullata* with nuclei in process of division. *B*–*D*, Fragments of threads of *Calothrix pulvinata* showing nuclear division.

In other microörganisms the nucleus is much more complex. Yet frequently this nucleus is found in a primitive state quite different from typical nuclei of higher organisms. In some amœbæ, the nucleus is formed simply of a poorly defined membrane filled with nuclear fluid, and a large body of chromatin resembling a nucleolus called the *karyosome* or *centriole-nucleolus* (Fig. 21), because it acts both as a centriole and as a nucleolus. In the center of the karyosome is frequently seen a more intensely chromophilic corpuscle corresponding to the centriole (Fig. 20, *B*, *a*).

Many protozoa and some algæ have a centriole-nucleolus, but it is wholly enclosed in the nuclear fluid. The chromatin appears as little grains or as a network (Fig. 20, *A*, *a*).

In the higher microörganisms (protozoa and fungi) the nucleus

begins to take the form of typical nuclei. The centriole detaches itself from the karyosome which becomes a true nucleolus, and may remain either wholly intranuclear (Fig. 19, *A*, *a*, 21, *A*, *a*), or become entirely extranuclear (Fig. 19, *B*, *a*, 21, *B*, *a*).

Theory of Binuclearity of Cells and Chromidia.—In the infusoria, the nuclear structure divides into two nuclei (Fig. 7); a large one, the *macronucleus* or *vegetative nucleus*, which functions during the vegetative life of the cell, and a small one lodged in a hollow of the macronucleus, the *reproductive nucleus* or *micronucleus*. At fertilization, the macronucleus is disorganized and its place taken by the micronucleus which reproduces by division both a micronucleus and a macronucleus. Certain flagellates have likewise two nuclei, a large vegetative and reproductive nucleus, and a small *micro-* or *kinetonucleus* which controls the formation of the flagellum.

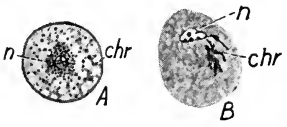


Fig. 6.—Chromidia in protozoa. *A*, The cycle of the microgamete of *Coccidium schubergi*. (*After Schaudinn.*) *B*, *Entamœba hystolytica*. (*After Hartmann.*) *n*, Nucleus, chr. chromidia.

Starting from these facts, a few investigators have tried to demonstrate that all cells have two nuclei. Recent evidence reveals that there are in the cytoplasm of most protozoa small chromophilic granules, like the chromatin material, which are supposed to emigrate from the nucleus during certain phases of development, and which are likened to the nuclear substance (Fig. 6). These granules are called *chromidia*, and all the granules scattered in the cytoplasm are designated as the *chromidial structure* or *chromidium*. Chromidia have been found in the cells of higher organisms. There is a theory that this chromidial system represents a second nucleus, the vegetative nucleus, scattered in the cytoplasm, and that the entire cell is provided with two nuclei, one of which has passed unseen up to this time because of its diffuse form. This theory is much doubted to-day, and it seems probable that the chromidium is simply a reserve material for the cell, or corresponds to formations which will be described later as *mitochondria*.

Cytoplasm.—*Appearance and Properties of Cytoplasm.*—Cytoplasm may be defined for our purposes as a semi-fluid substance, granular in appearance, and reacting with an acid stain. It has three essential physiological properties, nutrition, motility, and sensibility. Cyto-

plasm appears to be composed largely of protein substances and of diverse lipoid substances in a state of colloidal solution. It varies widely according to circumstances, consequently it may be useless to search for any definite structure. In many microörganisms, as for example the protozoa, there is on the periphery of the cell a hyalin zone which is called the *ectoplasm* to distinguish it from the rest of the cytoplasm, the *endoplasm* (Fig. 16).

Chondriosomes.—Recent research has demonstrated special functioning bodies in the cytoplasm, the *mitochondria*, which seem to be the constructive elements of cytoplasm. They are a part of its structure, and are supposed to play an important physiological rôle in the cell. These structures, visible in the living organism, but stained

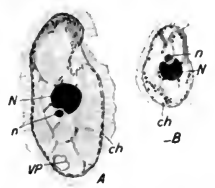


FIG. 7.—*Glaucoma piriformis*, infusorian with (*N*) macronucleus, (*n*) micronucleus (*ch*) mitochondria, (*vp*) pulsating vacuole. (*After Fauré-Frémiet.*)

FIG. 8.—Division of micronucleus and of the chondriosomes in *Carchesium polypinum*, infusorian. (*After Fauré-Frémiet.*)

only by a special process, are sometimes in the form of small isolated granules (*granular mitochondria*, Fig. 7, *B*), or of small threads (*thread-mitochondria*) or sometimes of rods much like certain bacilli (*rod-mitochondria*, Fig. 7, *A*). These forms frequently change from one to the other. The granular mitochondrium is able to elongate itself into a rod which is itself capable of dividing up into thread-mitochondria. All the mitochondria of one cell are called the *chondrium*. These structures seem to be made up of lipoidal substance and phosphates of albumin.

The mitochondria cannot generate themselves directly from the cytoplasm, but are formed always from preëxisting mitochondria by division. They apparently transmit themselves, after having divided, from the egg to the adult individual, and from the adult individual to the egg (Fig. 8).

Physiologically, mitochondria are organs of elaboration. In them, through some unknown physico-chemical phenomena, most of the products of cell activity may be formed. The product, whatever may be its specific nature, has its origin in a granular mitochondrium or in a rod-mitochondrium. It is surrounded by a mitochondrial exterior surface inside of which it develops slowly; the exterior surface remains until the product has reached its state of maturity.

It has been known for some time that there exist in higher plants corpuscular elements called *plastids* or *leucoplastids*, which also possess a synthetic function. Some, the *chloroplastids*, make the chlorophyl

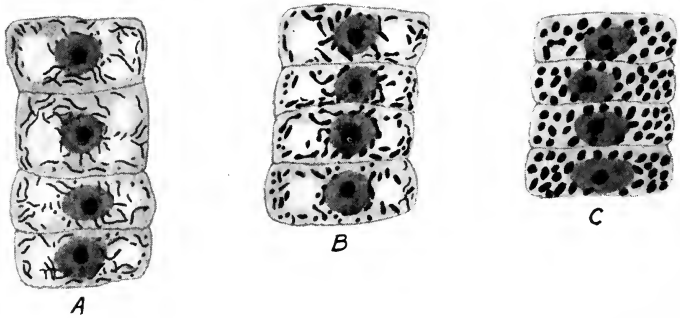


FIG. 9.—Formation of chloroplasts in the young leaf of barley. *A*, Very young cells in which appear rod-mitochondria. *B*, Older cells in which the rod-mitochondria are transforming themselves into chloroplasts. *C*, Cells in which the chloroplasts are definitely constituted.

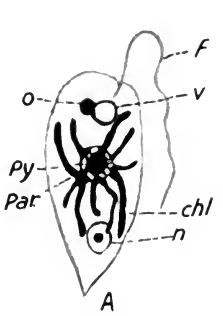
which, by using rays of light as energy, forms starch; others, the *amyloplastids*, confine themselves to forming starch from the excess sugars found in the cells; still others, the *chromoplastids*, constitute the pigment bodies of plants (xanthophyl, carotins). It has been recently shown that plastids are nothing but mitochondria which have undergone greater differentiaton and specialization than those which, at the expense of ordinary mitochrondria derived from the egg, have increased in size (Figs. 9, 10).

Mitochondria have been found in most protozoa and fungi. In the latter they take part in the formation of reserve products, especially the *metachromatic corpuscles* of which more will be said later.

Mitochondria are most highly developed in algæ where they give origin to chloroplastids as in higher plants. On the other hand, in

the lower forms, no mitochrondria seem to exist, but the chloroplastids take on certain special characteristics. Instead of small scattered corpuscles is found one, or occasionally several, large chloroplastids filling most of the cell. They are in various shapes—ribbons, spirals, nets, etoilated bodies (Fig. 11), etc.—but all appear to be made up of a mitochondrial substance. Their physiological rôle is much more general than in the chloroplastids of higher plants. They produce not only the chlorophyl, but other pigment bodies, the starch or paramylum, metachromatic corpuscles, and globules of fat. Conse-

FIG. 10.



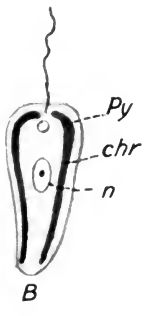


FIG. 11.

FIG. 10.—A cell from the root of a bean in which the rod-mitochondria (*ch*) form in the course of their development amyloplasts from (*p*) which spring grains of starch (*a*).

FIG. 11.—*A*, *Euglena viridis* with its star-like chloroplasts (*chl.*) at the center of the organism, the pyrénoid body (*Py*) surrounded by grains of paramylum (*Par*), eye-spot (*o*), contractile vacuole (*v*), flagellum (*f*), nucleus (*n*). (*After Dangeard.*) *B*, *Microglena punctifera*, with two elongated chromatophores arranged longitudinally. (*After Stein.*)

quently the complex chloroplastids of the algæ with their general function have been considered as a special form of chondrium which, instead of being scattered in the cytoplasm as a number of small structures, finds itself gathered in very compact masses.

The *Cyanophyceæ* are the only microörganisms in which the chondrium has not been found. In the *Cyanophyceæ* the chlorophyl and the blue pigment (phycocyanin) associated with it are diffused throughout the cytoplasmic area surrounding the nucleus. The very primitive structure of the algæ explains to some extent this absence of an important structure of the cell.

Vacuoles.—There is always in the cytoplasm one (or several) rather bulky vesicle filled supposedly with an aqueous solution of mineral salts called a *vacuole.* Vacuoles play an important part in the absorption of liquids by the cell. Owing to the mineral salts dissolved in the vacuole-fluid, the concentration of which is ordinarily higher than that of the surrounding medium, the vacuoles become the center of osmotic forces which consequently cause a part of the ambient liquid to penetrate the cell and determine its turgescence.

Very curious vacuoles are found in many protozoa, namely, the *pulsating vacuoles* (Figs. 7, 11). They are small vacuoles which expand and contract rhythmically, and which are considered as excretory and respiratory organs. The water that has entered the cell gathers in this vacuole and is expelled as it contracts. Probably in crossing the body this water yields its oxygen to the cytoplasm in order to charge itself with carbonic acid and the products of metabolism.

Reserve Products.—The cytoplasm encloses some structures differentiable by means of certain stains or chemical reagents as granulations, but which are not constituent elements of cytoplasm; they come from a secretion of the cytoplasm, and only under certain conditions. These grains may be found either in the cytoplasmic substance itself, or in the vacuoles included in the cytoplasm. Most of these granules are reserve products which appear when nutrition is deficient. Among the reserve products most common in microörganisms are the granules called *metachromatic corpuscles* (Fig. 11, *A*). These bodies, which are the object of a special study in connection with molds and yeasts, are made up of a substance the nature of which is still unknown, and are found in nearly all fungi, in most algæ and bacteria, and in many protozoa.

Glycogen and paraglycogen are equally well distributed in microörganisms (fungi, protozoa). Among algæ, glycogen is found only in the *Cyanophyceæ,* but it is elsewhere replaced by starch or paramylum (Fig. 10), common products of chlorophyllic assimilation.

There are also the protein substances, such as crystalloids of *mucorin* scattered in the *Mucorinæ,* or the globules of fat common in all cells (Fig. 12, *B*).

Most of these substances seem to result from the activity of the chondrium structure. Recent investigation shows that the metachromatic corpuscles have their rise among the mitochondria. It

has long been known, on the other hand, that the starch and paramylum are always formed in the chloroplastids.

MEMBRANE.—The cell is usually enveloped in a more or less heavy membrane, secreted by the cytoplasm, which acts as a protective organ for the cell.

The presence of the membrane is not, however, indispensable; many protozoa do not have it, and are consequently naked cells. Motility in many microörganisms is closely associated with the membrane, for the movement of cytoplasm and the flexibility of the mem-

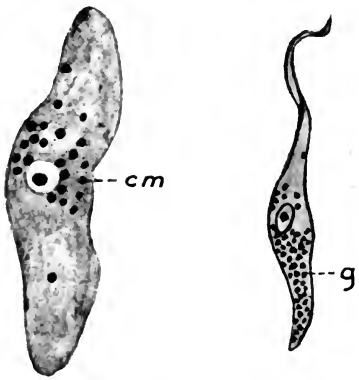


FIG. 12.—Metachromatic corpuscles (*cm*) in *Sarcosporidia, Sarcocystis tenella.* (*After Erdmann.*) Fat globules (*g*) in *Trypanosoma rotatorium.* (*After Doflein.*)

brane are essential factors. Cells as a rule have a membrane of different degrees of thickness and composition. It may be albuminoid or chitinous (*Infusoria*), or it may be made up of carbohydrates, as cellulose, pectose, and callose (algæ, fungi). Bacteria always have a membrane, but its nature has not yet been definitely determined. Often the cell membrane is able to thicken noticeably, and thus protect the cell from environing influences; the cell may then be regarded as transformed into a cyst which passes into a state of sluggish existence. Encystment is frequent with protozoa, and is produced when the environment becomes unfavorable (Fig. 13, *A*).

The external layer of the membrane frequently undergoes modifications, transforming itself into a mucilaginous or gelatinous sub-

stance as we see in many *Cyanophyceæ*, in bacteria surrounded by capsules, and in zoöglea. The membrane then becomes extremely thick (Fig. 13, *B*).

Locomotive Structure.—Most algæ and fungi cannot move. Many bacteria and all protozoa have more or less perfected locomotive structure.

The *Cyanophyceæ* and many bacteria, although without locomotive organs, present nevertheless oscillatory movements which seem due to a general movement of the cytoplasm translated exteriorly because of the flexibility of their membrane. With these exceptions, movement is effected by means of a locomotive structure.

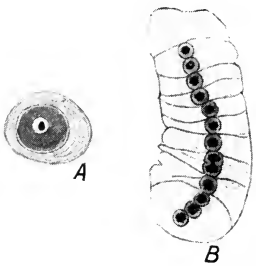


Fig. 13.—*A*, Cyst of *Amœba limax*. (*After Dangeard.*) *B*, Thread of nostoc surrounded by a thick mucilaginous case.

This structure is found in its simplest form in the *pseudopodia* of the amœba. The naked cell of the amœba pushes out pseudopods, simple expansions of the ectoplasm arising at any part of the body, which take various shapes, and reënter the body without leaving the least trace of their existence. It is a result of motility of the cytoplasm, one of its essential properties, shown here exteriorly because of the absence of a cellular membrane.

The locomotive structure is more complex in other protozoa; the pseudopod is replaced by contractile appendages—*flagella*, or *vibratile cilia*.

The flagellum is a contractile appendage of definite shape and position which draws the body after it by means of waving movements. It is found on bacteria and flagellates.

The organ of locomotion of bacteria is still little known (Fig. 14). It consists of a certain number of contractile appendages placed at one end of the cell, or at both, or sometimes distributed over the whole body. These appendages, called vibrating appendages, have many of the characteristics of flagella. Their existence, for a long time doubted, is now pretty well established.

The locomotive structure of the *Flagellata* is much better known. It is characterized by one or more flagella inserted in the anterior extremity of the cell. In case of more, one frequently folds back

toward the posterior end. In the lateral region of the cell it unites with a contractile membrane, the *undulating membrane*, running in spiral form along the length of the body, of which it is the free end. Flagella are made up of one or more elastic fibers, surrounded by a thin cytoplasmic sheath.

The vibrating cilia are also contractile appendages, differing from the flagella only in their smaller size. They cover the whole body of the cell, as in the case of infusoria, enabling them to move about very easily in liquids.

Certain facts lead us to believe that flagella are only transformed pseudopods in which the cytoplasmic structure has changed and at the same time the kind of movement. Thread-like pseudopods are found with a rapid rhythmic movement which may serve as intermediate forms. Be that as it may, the method of forming these organs is of special interest. Apparently they are formed under the influence and at the expense of the centriole.

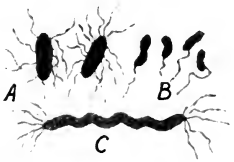


FIG. 14.—Organs of locomotion in bacteria. *A, B. subtilis.* (*After Fischer*) *B, Microspira comma.* (*After Fischer and Migula.*) *C, Spirillum rubrum.*

In the *Flagellata* the flagellum is always inserted in the centriole or in a similar organ which appears to issue from the centriole. It is not rare to find in cellular division some cells in which the nucleus is dividing with a centriole at each of its poles. Each serves as a point of insertion for a flagellum (Fig. 15, *A*, *D*, *E*).

According to recent works, the flagellum is formed in general in one of two somewhat different methods.

In the first case, the centriole divides itself by an elongation, followed by a contraction into two centrioles which remain united to each other by means of a fine thread, the *centrodesmose*. The centrodesmose then elongates, dragged by the centrioles, as far as possible from the nucleus, and transforms itself into a flagellum.

In the second case, the centriole divides itself a first time just as in the preceding case, but the centriole farthest from the nucleus immediately undergoes a second division, thus making three centrioles. The one nearest the nucleus remains a centriole during nuclear division. The centriole situated somewhat farther from the nucleus becomes the point of insertion for the flagellum, and is called the *blepharoplast* or *basal*

grain. The centriole is united to the blepharoplast by a *centrodesmose*, the *rhizoplast*, which is often absorbed. Finally, the last centriole situated beyond the blepharoplast about equally distant, also unites with this cell-organ by a centrodesmose and, by approaching the extremity of the cell, causes the elongation of the centrodesmose which transforms itself into a flagellum.

In the infusoria the vibratile cilia insert themselves in the ectoplasm and pass through the cuticle to reach the exterior. At the point of

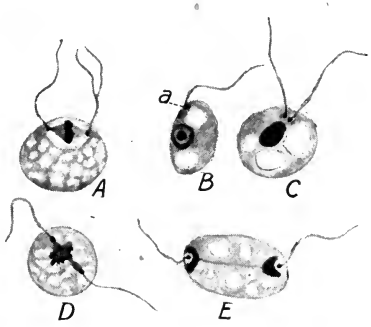


FIG. 15.

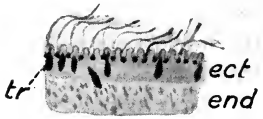


FIG. 16.

FIG. 15.—*A*, *Spongomonas uvella.* The nucleus is undergoing mitotic division. Two centrioles, each at the base of a flagellum, are located at the two extremes of the spindle. (*After Hartmann and Chagas.*)

B, *Monas termo.* The cell lies in repose; a centriole (*a*) lies at the base of the flagellum; in (*C*) there are two centrioles, in (*D*) the two centrioles occupy the two poles of the nucleus during the process of mitosis; in (*E*) exists the final nuclear division. (*After Martin.*)

FIG. 16.—Fragments of the peripheral portion of *Prorodon teres* (infusorian) with vibratile cilia and their basal corpuscles. (*ect*) Ectoplasm; (*end*) endoplasm; (*tr*) trichocysts. (*After Maier and Gurwitch.*)

insertion of each of these cilia is a small chromatic corpuscle or basal grain, a *trichocyst*, also supposed to arise from a repeated division of the centriole (Fig. 16).

The centriole which, as we shall see later, seems to be a motor organ directing the internal cytoplasmic movements during cellular division, appears also to be a motor organ of the external movement of the cell.

Reproduction of the Cell

Various Processes of Reproduction.—Reproduction of microbes is affected by various processes; the cell may reproduce itself by transverse or longitudinal fission, binary division, schizogony (bacteria, flagellata, molds, Figs. 4, *A;* 17; 19, *A*). This is by far the most frequent. It sometimes, however, divides itself by *budding, gemmulation* (Yeast, Fig. 2); that is, by the formation of a small protuberance which separates itself from the mother cell as a small daughter cell which, once free, grows slowly to maturity.

Finally, a last process and a very frequent one is the formation of internal spores, or *sporogony* (Fig. 18). The nucleus undergoes a

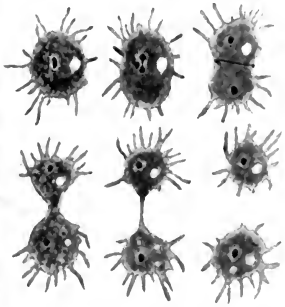


Fig. 17.—Schizogony in *Amœba polypodia* with amitotic division of the nucleus. (*After Schulze and Lange.*)

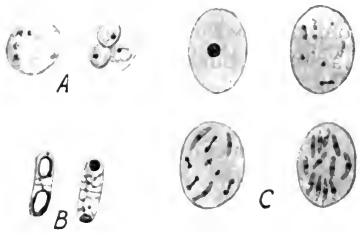


Fig. 18.—Sporogony. *A*, Formation of spores in *Saccharomyces cerevisiæ*. *B*, Formation of spores in *B. mycoides*. (*After Guilliermond.*) *C*. Formation of spores in *Leucocytozoon lovati*. (*After Fantham.*)

certain number of divisions, and the cytoplasm divides itself inside the cell in as many small cells as there are nuclei. These cells become spores and are set free by a rupture in the wall of the mother cell. Sometimes all the cytoplasm of the mother cell divides into spores, and sometimes only a part of the cytoplasm is used, the rest *epiplasm* serving as nourishment to the spores during their growth.

Whatever the means by which the cell reproduces itself, cytoplasmic changes and nuclear changes take place at the same time. The most important of the cytoplasmic changes is the distribution of the chondrium structure between two daughter cells, often preceding the division of this cytoplasmic structure (Fig. 8).

The nuclear phenomena are much more important, and better known. The nucleus divides in order to furnish each daughter cell with a nucleus containing the same amount of chromatin.

NUCLEAR DIVISION.—Nuclear division may occur in one of two ways, one very complex, (1) the *indirect mode, karyokinesis* or *mitosis;* the other very simple, (2) the *direct mode,* or *amitosis.*

Indirect Division, Karyokinesis, or Mitosis.—We shall begin with the indirect mode which is by far the more common, using as an example a *Heliozoön,* the *Acanthocystis aculeata* (Fig. 19, *A*). The nucleus of this protozoon at rest contains a large karyosome of a spongy structure, and a chromatic network. Outside the karyosome in the nuclear vesicle is a centriole surrounded by a hyaline zone, the archoplasm (Fig. 19, *A*, *a*).

Mitosis may be divided into four steps or phases.

The *first phase* or *prophase* begins by the emigration of the centriole from the nucleus outside of which it surrounds itself by cytoplasmic irradiations, making a star-like body, called the *aster* (Fig. 19, *A*, *b*). Following this, the karyosome dissolves in the nucleoplasm, supposedly conveying material to the chromatic network which enriches itself noticeably in chromatin. The chromatic network then relaxes, thickens and transforms itself into a more or less spiral cluster, the *spireme* (Fig. 19, *A*, *c*). At the same time the centriole divides into two centrioles, each surrounded by an aster (Fig. 19, *A*, *c*). Soon these centrioles place themselves at the two opposite poles of the nucleus (Fig. 19, *A*, *d*), while the spireme breaks itself up into a definite number of chromatic sections, the *chromosomes.* While this is taking place, the nuclear membrane dissolves itself into a series of cytoplasmic fibrils, the *achromatic spindle,* resistant to nuclear stains. They appear in the middle of the nucleus and converge at each end to the centrioles (Fig. 19, *A*, *d*, *c*). The chromosomes group themselves in the center of the spindle as the *equatorial plate* (Fig. 19, *A*, *e*), the formation of which completes the prophase. Each of the chromosomes is attached to one of the fibrils which make up the achromatic spindle.

The *second phase* or *metaphase* consists of the longitudinal division of the chromosomes each of which divides itself into two equal chromosomes.

In the *third phase* or *anaphase* the chromosomes equally divided

move to the two poles where they make two polar plates. The centrioles located here seem to have some attraction for the chromosomes.

Finally comes the *telophase* or phase of reconstitution of the two nuclei which terminates the process. In this phase, the chromosomes

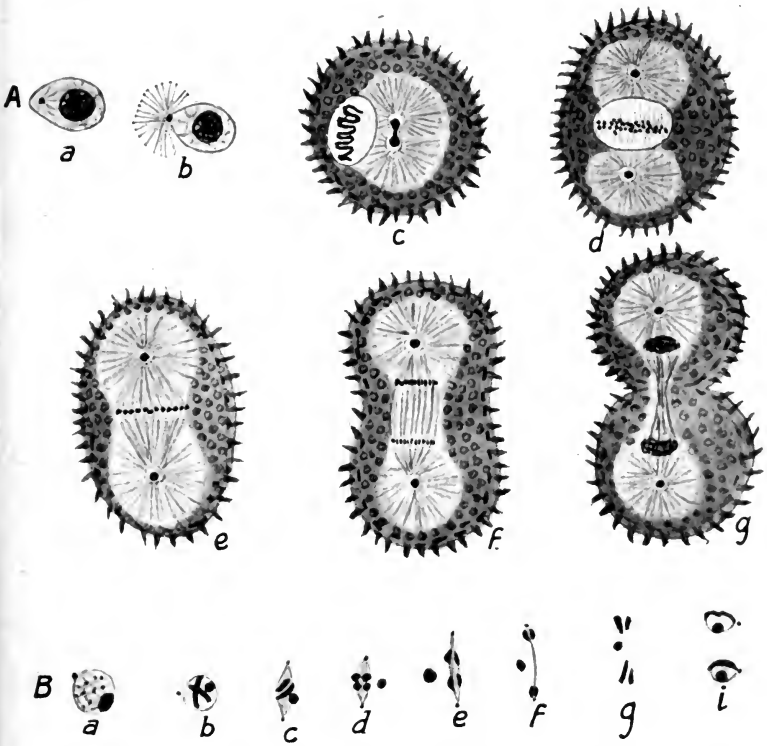


FIG. 19.—Karyokinesis (metamitosis). *A*, *Acanthocystis aculeata;* (*a*) nucleus in state of repose with an intranuclear centriole; (*b*) (prophase) the centriole moves to the periphery and out of the nucleus and forms an aster (*After Hertwig*); (*c*) the division of the centriole and spireme; (*d*) the formation of the equatorial plates and the achromatic spindle; (*e*) equatorial plates; (*f*) anaphase; (*g*) telophase. (After Schaudinn.) *B*, In *Coleosporium senecionis* (Uredineæ). (*a*) Nucleus at rest with its centriole extranuclear; (*b*) formation of chromosomes; (*c*) equatorial plate; (*d*) metaphase; (*e*) anaphase; (*f*) (*g*) (*i*) telophase. (*After Madame Moreau.*)

form a spiral chromatic cluster making a spireme at each of the poles (dispireme stage, Fig. 19, *A*, *g*); each of the spiremes is then surrounded

by a nuclear membrane in which is included the centriole. Thus the two nuclei are formed in which a nucleolus soon appears. Meanwhile the cell has elongated, become constricted in the center, and finally broken into two cells (Fig. 19, *B*, *f*, *g*, *i*). The achromatic spindle completely disappears.

This method of division represents the typical method of karyokinesis, that which is observed in higher organisms with the single difference that the centriole is intranuclear, whereas in the cells of higher organisms it is ordinarily outside the nucleus in contact with the nuclear membrane. An analogous mitosis is found in the *Uredineæ* (Fig. 19, *B*, *a*, *i*), except that the centriole is here found to be extranuclear (Fig. 19, *B*, *a*), the asters are lacking, and the nucleolus persists to the end of mitosis expelled in the cytoplasm. The physiological significance of the nucleolus in this case is not known. This method of division is seen in certain molds and higher protozoa, and is called *metamitosis* or *perfect mitosis*.

Summing up, mitosis is a process functioning to make an absolutely equal division of the chromatin between the two nuclei. This distribution is performed by the breaking up of a spireme into a definite number of chromosomes, a number varying according to the species but always constant for any single species, and then by a longitudinal division of the latter. The centrioles seem to play an important rôle in this phenomenon, in directing it, and in attracting the chromosomes once divided toward the poles of the cell where the nuclei are formed.

It is not necessary to conclude that the processes of mitosis are as complex as in other microörganisms. Relatively simple in the lower forms, mitosis becomes complicated as it climbs the ladder, gaining the characteristics of metamitosis only in the most advanced forms.

The simplest case is found in the *Cyanophyceæ* (Fig. 5). Here cellular division begins by the outline of the transverse partition which appears in the form of a peripheral ring. At the same time the chromatic network takes a definite arrangement; its filaments arrange themselves parallel to the longitudinal axis of the cell, thus giving this division the appearance of a mitotic division. The outline of the partition extends little by little toward the middle of the cell, leaving open only a small spherical space in its center to which the fibers of the network then contract, and the nucleus takes the form of

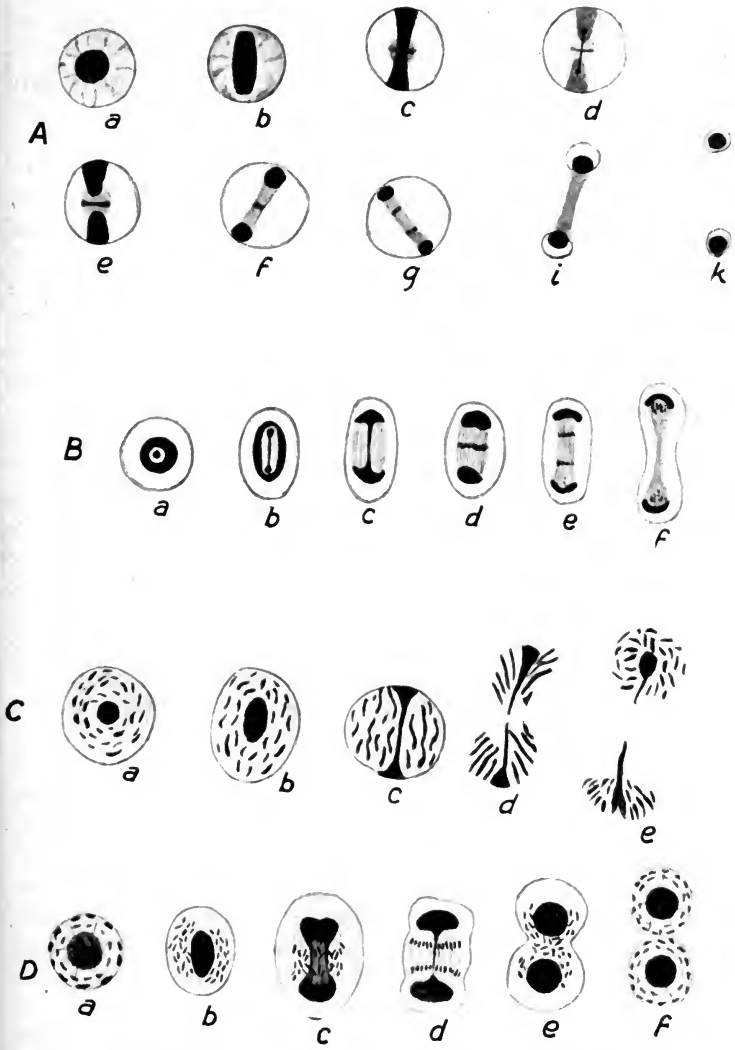


FIG. 20.—Protomitosis. *A*, In *Amœba mucicola*. (*a*) Nucleus at rest; (*b*) beginning of prophase; (*c*) division karyosome; (*d*) division of centriole; (*e*) (*f*) equatorial plate; (*g*) metaphase; (*i*) (*k*) telophase. (*After Chatton.*) *B*, In *Amœba froschi*. (*After Nägler.*) *C*, In *Euglena splendens*. (*After Dangeard.*) *D*, In *Amœba diplomitotica*. (*After Beaurepaire Arago.*)

a dumb-bell. Soon the partition stops completely, the filaments of the contracted part of the nucleus break up and the two daughter cells appear separated by a partition. The two nuclei whose filaments have been sectioned by the partition are not slow in recovering their integrity (Fig. 5, *b*).

We find in the *Amœba mucicola* (Fig. 20, *A*) a much more characteristic mitosis, though more primitive. The nucleus of this amœba when at rest is made up of a nuclear fluid surrounded by a membrane in which are a large karyosome and some small grains of chromatin localized on the periphery (Fig. 20, *A*). In the center of the karyosome is a small chromophilic centriole. The prophase begins by the elongation of the karyosome to a rod-shaped body (Fig. 20, *A*, *b*) which then transforms itself into a dumb-bell (Fig. 20, *A*, *c*). The centriole also elongates and becomes constricted in the center (Fig. 20, *A*, *d*). At the same time an achromatic spindle appears all about the constricted region of the karyosome in the middle of which the grains of chromatin group arrange themselves peripherally to form an equatorial plate, but there is no differentiation of this chromatin into two chromosomes (Fig. 20, *A*, *c*, *d*). In the metaphase the karyosome and the centriole divide into two polar masses (Fig. 20, *A*, *e*, *f*), the equatorial plate separates into two plates which, in the anaphase, emigrate to the poles (Fig. 20, *A*, *g*) drawn by the centrioles. In the telophase the spindle elongates, disappears, and the two nuclei are formed at the poles (Fig. 20, *A*, *i*, *k*). The nuclear membrane exists during the entire phenomenon.

In other microörganisms (*Amœbæ*, *Flagellata*, *Euglenæ*) is found a similar mitosis except that the chromatin distributed in the resting nucleus as a network or as rod-shaped bodies forms an equatorial plate made up of true chromosomes (Fig. 20, *B*, *C*).

Another form of mitosis, *promitosis*, is characterized by the fact that the centriole is included in the karyosome, by the persistence of the nuclear membrane, and by the simultaneous division in the metaphase of the karyosome and of the chromatin gathered in an equatorial plate.

Between promitosis and metamitosis are a series of intermediate forms. In the *Pelomyxa palustris*, for example, the centriole while remaining intranuclear is able to separate itself from the karyosome (Fig. 21, *A*, *a*). The prophase here begins with the usual division of

the centriole (Fig. 21, *A*, *b*, *c*), and the two resulting centriole-threads pass to the extremities of the achromatic spindle, while the karyosome coöperates in the formation of the chromosomes (Fig. 21, *A*, *d*, *e*).

In other cases (various fungi, *Gregarinæ*, etc.), the centriole becomes extranuclear, and the karyosome acts as a true nucleolus (Fig.

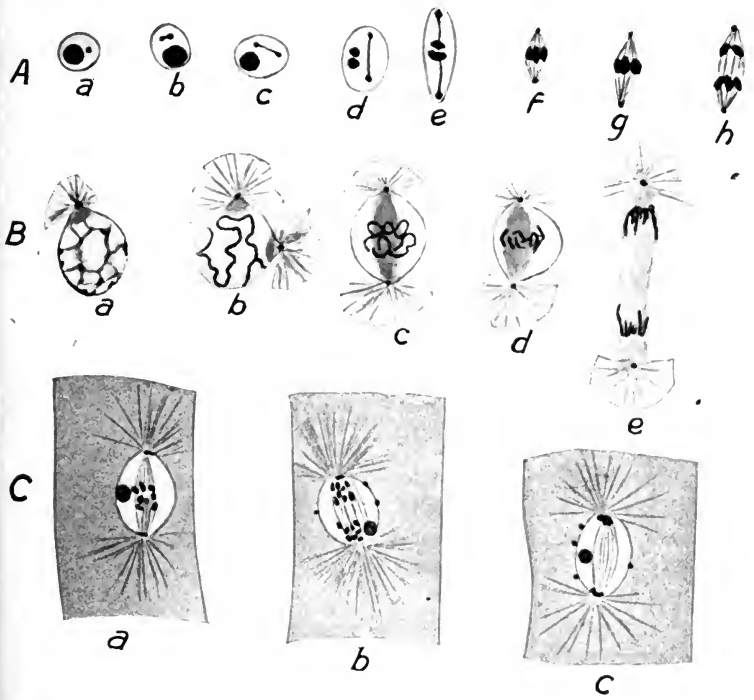


FIG. 21.—Mesomitosis. *A*, In *Pelomyxa palustris*. (*a*) Nucleus at rest; (*b*)—(*e*) division of centriole; (*f*) (*g*) equatorial plate; (*h*) anaphase. (*After Bolt.*) *B*, In *Urospora lagidis* (*Gregarina*). (*a*) Nucleus with extranuclear centriole and aster; (*b*) the centriole is divided and the spireme is formed; (*c*) spireme; (*d*) equatorial plate; (*e*) anaphase. (*After Brasil.*) *C*, In the ascus of *Galactima succosa* (*Ascomycete*). (*a*) Equatorial plate; (*b*) anaphase; (*c*) telophase.

21). Sometimes it dissolves at the beginning of mitosis, seeming to aid the development of the chromatin of the spireme, and sometimes it persists during the entire process and is expelled in the cytoplasm at the end of the phenomenon without any known function. The

name of *mesomitosis* has been given to all the mitoses which distinguish themselves from promitosis by the persistence of a nuclear membrane throughout the phenomenon.

Direct Division or Amitosis.—This consists simply of an elongation of the nucleus followed by a median constriction, then by a rupture of this constricted part without an equal division of the chromatin between the two nuclei which often are not the same size. It is a simple breaking up of the nucleus. Amitosis, then, does not necessarily insure the equal distribution of chromatin between the two nuclei. This rare process is found in higher organisms only in old cells that are degenerating, or in diseased cells. Although for a long time it was thought to be a primitive phenomenon, it is now considered to be degenerative. We see, however, in certain *Amœbæ* and *Mycetozoa* the karyosome enclosing all the chromatin divides itself into two equal

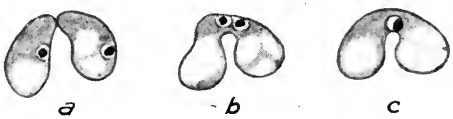


FIG. 22.—Conjugation in *Schizosaccharomyces octosporus*. (*a*) Two gametes in the process of fusion; (*b*) (*c*) nuclear fusion.

bodies, showing the characteristics of a very primitive mitosis (Fig. 17). Amitosis seems to exist normally in yeasts and in certain molds. In the yeasts, for example, the nucleus divides by amitosis in the course of budding (Fig. 2), and mitosis is found only in the course of sporulation.

SEXUAL CHANGES.—In most microörganisms at certain times during their existence occur sexual changes, or fertilization, which seem to give them a new strength. It is followed by a period of very active reproduction, whence the name of sexual reproduction given to these changes. This consists essentially in the fusion of two equal *isogamous* (*isogamy*) or unequal, *anisogamous* (*heterogamy*) cells or *gametes*. In the latter case, the male is small and active, and the female large and passive. The fusion between the two cytoplasms and the two nuclei takes place at the same time (Fig. 22).

If nuclear fusion were not compensated by an elimination of chromatin, the nucleus would increase in this substance at each fertilization. But this change is succeeded immediately in protozoa by a

common process called *chromatic reduction*. The chromosomes in the course of the divisions which precede the formation of the gametes reduce themselves to half by a complex process which it would be superfluous to describe here. The same chromatic reduction takes place in the fungi and algæ, but this does not always precede fertilization. It may follow it immediately as in the yeasts where it seems to produce itself during the nuclear divisions in the ascus. It may also occur during other stages of development.

CHAPTER II*

MOLDS

Fungi in General

A sharp line cannot be drawn between the bacteria and the fungi. Certain border groups such as *Leptothrix* and *Actinomyces*, filamentous forms in which branching and even the production of differentiated spores occur, are sometimes described as bacteria and sometimes as fungi. From the microscopic point of view, forms in which the cells can be handled as bacteria by cover-glass staining may be conveniently treated by bacteriologists. Forms in which the cells are larger, with definite walls, vacuoles, and cell sap, in which the cells collapse when dried and lose their distinguishing characters, may be better treated as fungi. No rule holds for all groups.

With some exceptions, there is, among the cells of the true fungi, a differentiation of function into vegetative or assimilative cells and reproductive cells. The fungous body is usually composed of threads (technically called *hyphæ*, singular, *hypha*). These *hyphæ* usually branch in more or less complex manner forming networks or webs, collectively called *mycelium*. Hyphæ may be one-celled or composed of many cells placed end to end as shown by the cross walls, called *septa*, seen in them. These threads grow either by the formation of new cells at the growing tips (called *apical growth*) or by the division of cells in the hypha (*intercalary growth*). The fungous cells rarely divide in three planes to produce solid masses of cells. Both vegetative and reproductive masses are formed in great variety from such hyphæ. Often the thread-like character is almost or quite obliterated in the ripe masses, which may be fleshy, woody, carbonaceous, leathery and even horn-like in texture, as seen especially in the mushrooms, bracket-fungi, etc., but even in such cases the early stages show the structures to originate from masses of fungous threads.

* Prepared by Charles Thom. A. Guilliermond has furnished the section on "Cytology of Molds."

The formation of differentiated reproductive cells is, in general, characteristic of the fungi. The method of reproduction presents great variety. In the simplest forms, the reproductive cells are scarcely if at all distinguishable from the vegetative cells. In some species whole hyphæ break up so that each cell forms the starting-point of a new colony. Other forms develop special branches bearing reproductive cells. From these it is but a step to the production of fruiting branches, characteristic in form, called *conidiophores*, bearing cells markedly specialized as reproductive by form and frequently also by color, called *conidia*. These conidia are entirely asexual in origin and capable of growing directly into new colonies, although in many cases they are provided with resistant walls which enable them to live for long periods if conditions are unfavorable to growth at once. In other species, specialized resting cells with resistant walls are formed to enable the plant to survive unfavorable conditions. These are called *chlamydospores* or sometimes *cysts*. The name *gemmæ* is sometimes applied to similar structures, preferably to such as grow at once. The same end is reached in still other groups by the formation of *sclerotia* which are hard masses or balls of thick-walled cells filled with concentrated food materials. These sclerotia are frequently distinctive of the species producing them by size and appearance. They sometimes resemble the sexual fruiting masses. Resting structures of either type, especially when large, commonly produce typical spore-bearing structures at once after germinating. Many very complex fruit bodies such as the mushrooms appear to be entirely asexual in origin.

The systems of classification used are largely based upon the types of sexual fruit bodies produced. Where such fruit bodies are not known, the method of formation of the asexual spores furnishes the most satisfactory basis for grouping. In classifying fungi, certain types of spore formation are found to be characteristic of particular groups. Since within these groups various accessory types of fruiting occur, so that some species show three or even more forms of spores, that type of spore formation which is regarded as characteristic of the group is known as the perfect stage. If sexual fruits are found, these constitute the perfect stage of the group; if no such fruit is found, the most characteristic asexual form is used, as for example the common mushroom of commerce which is asexually produced so far as we know, yet represents the most perfect and most constant fruiting form produced

by a very large group. Between the typical forms are many gradations resulting in many families whose relationship to one or the other group is difficult to determine. Probably the ancestral history (phylogeny) of the fungi, if known, would show several or many lines of descent rather than one. Certain of these groups may be presented briefly.

BACTERIA.—In the scheme of plant grouping presented (page 77), which is only one of many attempts to show relationships, the bacteria are placed with a group of single-celled green or blue-green forms as *Schizophyta* or fission-plants because of reproduction only by the division of the cells.

PHYCOMYCETES.—The *Phycomycetes* are called algal fungi because they resemble certain groups of green filamentous forms in many particulars. In this group two general types of sexual reproduction are met with—zygospore formation and oospore formation. The first, found in the *Zygomycetes* represented by the common mucors, consists of the fusion of terminal cells of branches of the mycelium similar in appearance but differentiated in sex. As a result of this fertilization large thick-walled resting cells are produced, called *zygospores*, from a Greek root meaning yoked (Fig. 32). In oospore formation, found in the *Oomycetes*, the conjugating cells differ in appearance as well as in function. The *oospore* is large and is rich in food materials; the *antheridium* is much smaller, penetrates and fertilizes the egg, which afterward develops into a thick-walled resting spore. The very destructive downy mildews belong to this group.

ASCOMYCETES.—In this great group sexuality was denied until recent years, but has been proved in cases enough to establish a presumption of more general occurrence. The characteristic structure of the group is the *ascus*, a sac containing, when ripe, typically eight spores, sometimes a less number by the failure of some to develop, sometimes a larger number, usually some multiple of eight. The ascus when sexuality is known is developed subsequent to fertilization, not directly from an egg cell. The group presents a great variety of fruiting masses produced in connection with the asci. The simplest forms are loose webs of hyphæ enmeshing a few asci; other forms show clubs, cups, flask forms, crusted areas, the type of mass in each case being characteristic of the family, genus and species represented. Only a few of many thousands of these forms are encountered in bacteriological work.

One genus is, however, constantly found. The commonest species of *Aspergillus* produces bright yellow, globose fruiting bodies, called *perithecia*, filled with asci. These are borne upon the surface of the substratum and often give a yellow color to the colony by their abundance. Such perithecia consist of the ascogenous cells and the asci produced by them, about which a more or less completely closed sac or wall has been formed, by the development of the sterile cells adjacent to the fruiting ones.

Basidiomycetes.—In the *Basidiomycetes* there is still further reduction of the evidences of sexuality. In one border group, the rusts, sexual processes have been shown to be more or less developed. In the typical Basidiomycetes sexuality is reduced to a fusion of the nuclei in certain binucleate cells. The typical structure is the *basidium*, a spore-bearing cell characteristically producing at its apex four protuberances called *sterigmata* (singular, *sterigma*), each bearing a single spore. These basidia are grouped into many kinds of fruit bodies, from occurrence here and there upon a loose web of hyphæ to dense columnar areas covering the gills of the mushrooms or lining the cavities of the puffballs. Very few of these species occur in bacteriological studies.

Imperfect Fungi.—A very large number of species are known which have never been seen to produce the characteristic fruits of the great groups. These are brought together and described as form-genera by their method of asexual spore formation. From the lack of the organs used in classifying the other groups, these are called the *imperfect fungi* and their grouping regarded only as temporary, a convenience for the identification of materials. These include many forms of economic importance, and many of the species most frequently met in bacteriological work. Sometimes one species of a large group produces a perfect form while no other species can be induced to do so. Some of these species undoubtedly represent stages of perfect fungi whose perfect forms simply are not recognized as connected with these; others reproduce for an indefinite number of generations by conidia. Such cases do not appear to need the perfect form and hence apparently have, in some cases, lost the power to produce it.

As found in nature all these forms are parasitic, saprophytic, or capable of both modes of life. All depend more or less completely upon organic matter for nourishment. Great diversity exists, how-

ever, in their adaptation to environment. Many of them are not only parasitic but so closely adapted to parasitizing particular host-species as not to be found elsewhere. Others attack several or many species, usually related. Even among saprophytes many species are found only upon particular forms of decaying animal or vegetable matter. The great economic importance of these parasitic and closely adapted saprophytic species has been recognized by the development in recent years of the literature of plant pathology (phytopathology). These cannot be considered in this work.

Cytology of Molds*

General Structure of Molds.—Three kinds of cell-structure formation are found in molds:

1. Some, belonging to the *Phycomycetes*, show no cross-walls; they have a much branched, felted mycelium, but in the early stages there are no true transverse septa. Septa appear in many forms only when fruiting begins, but in the opinion of some they merely separate the living portions of the mycelium from those in which the cytoplasm is dead or degenerating. The cytoplasm in the unseptate mycelium forms one continuous mass; it contains a great many nuclei (Fig. 23, 1 and 2). Each nucleus with the cytoplasm surrounding it, according to Sachs, may be considered a physiological unit acting in a somewhat similar capacity as a cell, or may be designated as an *energid*. This view is not held by all observers, however. Considered thus, the mycelium represents the collection of a great many indistincts cell which are not separated by walls. The *Mucorineæ*, for example, belong to this structural type.

2. Other fungi, especially among the *Ascomycetes*, have a septate mycelium, but one in which the transverse septa do not restrict cellular functions as true cells. It consists of compartments containing a variable number of nuclei called *coenocytes* (Fig. 24, 1). Each compartment may be considered, not as a true cell, but as a colony of rudimentary cells, *energids*.

3. Still other molds have a mycelium consisting of true cells with a single nucleus, as for example *Endomyces fibuliger* (Fig. 24, 3 and 4) and *Endomyces decipiens*.

* Prepared by A. Guilliermond.

There are, moreover, molds which show both these last two structural types, with transitional forms between the two. For instance, in *Endomyces magnusii*, the mycelium, ordinarily consisting of areas, each containing many energids, can in some parts progress to a uninuclear cellular structure.

The conidia or spores of many molds may have either one or many nuclei, according to the

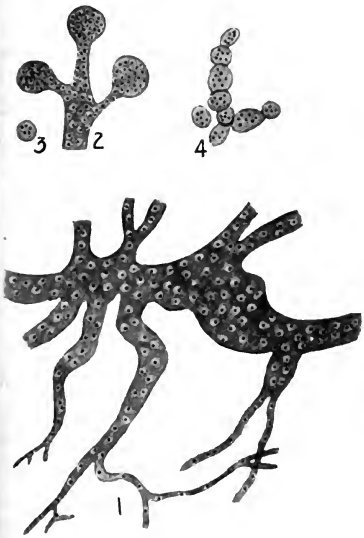


FIG. 23.

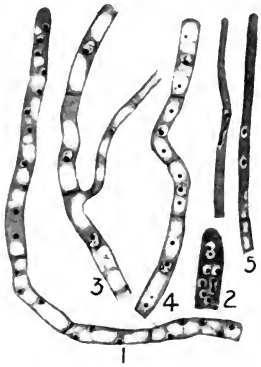


FIG. 24.

FIG. 23.—1, Part of the mycelium of *Thamnidium elegans* (*Mucor*). 2, Extremity of a filament of *Mucor circinelloïdes* showing three swellings about to form sporangia. 3, A spore of the same mold. 4, Yeast forms from the same mold. (*After Léger.*)

FIG. 24.—1, Mycelial filament of *Endomyces magnusii*. 2, Extremity of a filament of the same mold in the process of growth, with a dividing nucleus. 3 and 4, Filaments of *Endomyces fibuliger*. In 4, metachromatic corpuscles are seen in the vacuoles. 5, Filament on the way to increase, from the same mold, the nucleus dividing.

species. The spores of the *Mucorineæ* for example always have many nuclei (Fig. 23, 3); on the contrary, the ascospores of the Ascomycetes, the conidia of *Penicillium* and *Aspergillus*, contain generally but a single nucleus.

The yeast forms which result from the budding of the mycelium in some molds, most frequently have a single nucleus (Fig. 23, 4); however, in some, *Dematium*, are sometimes found yeast-forms containing

several nuclei. The yeast-forms of the *Mucorineæ*, which are not otherwise very typical forms, are always multinuclear.

To whichever of these three structural forms a mold belongs, it always represents some similar constitutional elements which we will now consider.

CYTOPLASM.—The cytoplasm is a semi-fluid mass, somewhat dense, sometimes homogeneous and containing a more or less considerable number of vacuoles. Certain methods of fixing and staining have recently made possible a demonstration, in the cytoplasm of the most diverse molds, of the presence of a *chondrium*, very clear and always splendidly exhibited. This consists mostly of fine rod-mitochondria, very long and flexible, generally lying parallel with the longitudinal axis of the cell (Fig. 25). Sometimes also it contains granular mitochondria.

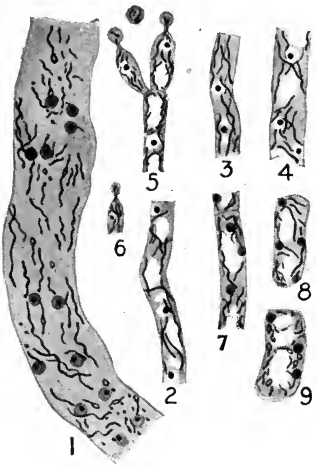


FIG. 25.—Various molds fixed and stained by a special technic, showing their chondrium. 1, Filament of *Rhizopus nigricans* (*Mucor*). 2–4, Filaments of *Penicillium glaucum*. 5 and 6, Fragments of the conidial organ of the same mold. 7, Filament of *Endomyces magnusii*. 8 and 9, Oidia of the same mold. In all these molds, chondrium is represented by long filaments, or sometimes by small grains. The filaments often show small vesicles at their crossing.

The cytoplasm also has reserve products, of which we shall speak later.

NUCLEI.—The nuclei show a differentiated structure which is sometimes difficult to demonstrate. They consist of a nuclear membrane, a hyaline nucleoplasm, a large nucleolus and a chromatic network. The last is sometimes indistinct, and it frequently happens that the nucleus appears to contain only a nucleolus; but a very careful examination always reveals the network (Fig. 24, 3 and 4).

The division of the nucleus is not always easy to observe. To study it, one must examine the growing tips of the mycelium. In some cases this consists in an elongation of the nucleus which soon assumes the form of a very slender dumb-bell which breaks apart at the narrow

portion. This is the extent of an amitotic or direct division (Fig. 24, 2 and 5).

Karyokinesis is usually seen only in the organs of fructification (asci, basidia, etc.); nevertheless, in the mycelium of the *Basidiomycetes* and *Mucorineæ*, true métamitoses have been found. In the *Mucorineæ* for example (Fig. 26), the nucleus loses its membrane (1–4) and gives rise to a spindle ending in a centrosome at either extremity, while two chromosomes form the equatorial plate at the center (5). Each of

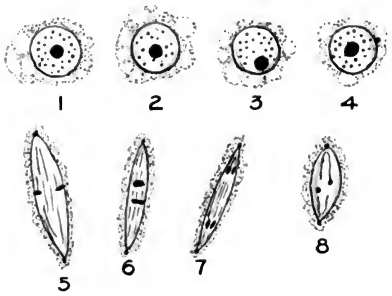


FIG. 26.—Nucleus of the *Mucor* (1–4), and various stages of its division (5–8). (*After Moreau.*)

the two chromosomes divides and the four resulting chromosomes are distributed between the two poles (6–8) where they form the two daughter nuclei (Moreau).

METACHROMATIC CORPUSCLES AND RESERVE PRODUCTS.—The vacuoles always contain a great many shining granules, showing Brownian motion and capable of being stained in the living state by neutral red and methylene blue. These bodies have staining qualities which permit them to be easily characterized. They are stained a violet-red by most of the basic dyes, aniline blue or violet. They also take on a very pronounced reddish tinge with hematoxylin (Fig. 27). By reason of this property of *metachromatism*, they have been called *metachromatic corpusles*. These bodies, which are very common in the *Protista*, have been found in yeasts, bacteria, algæ and protozoa. The chemical nature of the substance constituting them is still unknown, but the name *metachromatin* is often used for it.[1] Some authors, among

[1]Because of the priority and more exact signification, the names *metachromatic corpuscles* and *metachromatin* are preferable to the terms *grains of volutin* and *volutin* given by Arthur Meyer.

whom is Arthur Meyer, believe them to consist of a combination of nucleic acid, but this is a mere supposition.

On the other hand, the rôle of the metachromatic corpuscles is now well known. It is evident that they are reserve substances. Their evolution proves it. Thus metachromatic corpuscles appear in great abundance in the young asci of the higher *Ascomycetes* (Fig. 28, 1 and 2), then accumulate in the cytoplasm of *epiplasm* which is not utilized in the formation of the ascospores, gather all around the ascospores at the time of their forming (3–4), and are gradually absorbed by the latter in the course of their development (5). They therefore furnish

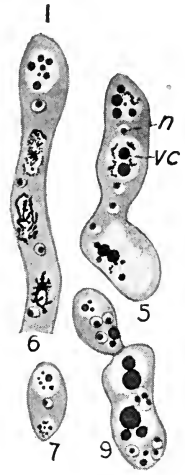


Fig. 27.

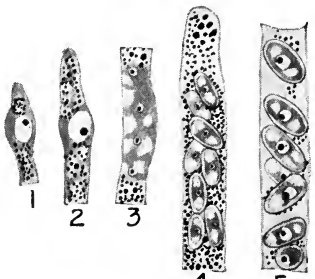


Fig. 28.

Fig. 27.—*Dematium species* Stained by a method permitting the differentiation of the metachromatic corpuscles. 1, Filament. 7 and 9, Yeast forms. 9, Yeast form starting to bud from mycelium. The metachromatic corpuscles are situated in the vacuoles in the form of small grains joined in chains (6) or isolated. Many appeer like large granules (9). *n.* Nucleus. *v.c.* Vacuole with metachromatic corpuscles.

Fig. 28.—Various stages of the development of the ascus in *Aleuria cerea.* 1 and 2, Young asci with their nucleus and many metachromatic corpuscles. 3, Fragments of an ascus after the second nuclear division. 4, Ascus, still young, in which the ascospores are surrounded by metachromatic corpuscles. 5, Older ascus in which most of the metachromatic corpuscles have been absorbed by the ascospores.

nourishment for the ascospores and from this standpoint behave exactly like glycogen and the globules of fat which are usually coëxistent with them in the cytoplasm. We shall see, moreover, that they undergo a similar evolution in the asci of yeasts. Likewise in the conidiophores of molds, notably in the fruiting heads of *Aspergillus* and

Penicillium, the metachromatic corpuscles are produced in great abundance (Fig. 29, 26 and 30), then gradually disappear as the conidia from (29, 3). Here again they serve as food for the conidia.

Metachromatic corpuscles appear not only in the vacuoles, but also in the perivacuolar cytoplasm. There they spring up, to diffuse finally in the vacuole where they increase. It is difficult to observe their manner of forming in the mycelial filaments, but in the preparation for sporulation some molds (asci of the higher *Ascomycetes*), it has recently been demonstrated that they start in the midst of the elements of the chondrium, which act as plastids similar to the plastids of the higher plants. They start in the interior of the granular-mitochondria or in the rod-mitochondria (Fig. 30). In the former case, a small corpuscle appears in the midst of a mitochondria, then develops gradually, while the mitochondrial membrane which envelops it grows thinner; is reduced to a small capping of the grain on one side; then disappears when the latter reaches maturity. It is noteworthy that the corpuscles emigrate with their plastid to the interior of the vacuoles during their development.

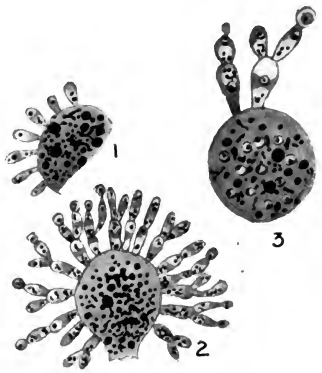


FIG. 29.—Conidial organ of *Aspergillus niger* with metachromatic corpuscles.

When the corpuscles start in a rod-mitochondrium, at the junction of these rod-mitochondria several small corpuscles are seen to form, then the parts of the rod-mitochondrium which join are absorbed and the corpuscles, enclosed in their mitochondrial membrane, once separated, undergo the same evolution as above.

Thus the metachromatic corpuscles, like grains of starch in the higher plants, start in the midst of the mitcohondria and develop gradually out of their mitochondrial matrix, and with the aid of the vacuolar substance.

In molds are found still other reserve products. One often sees globules of fat in the cytoplasm, which are easily stained a black-brown by osmic acid; and glycogen which can be differentiated by iodine

in iodide of potassium. The glycogen is contained in either the cytoplasm or the vacuoles. It is generally very abundant.

These products (fat and glycogen) undergo the same evolution as the metachromatic corpuscles, and they also accumulate in the organs of fructification (asci, conidial organs) to serve in the nourishment of spores and conidia.

FIG. 30.—Formation of metachromatic corpuscles in a cell of the perithecium of *Pestularia vesiculosa*. The rod-mitochondria form on their crossings vesicles (*c*) consisting of a metachromatic corpuscle unstained by the special method which served to differentiate the chondrium. Some corpuscles (*a*), more highly developed, are found in the vacuoles still surrounded by their mitochondrial shell; others (*c*) at the completion of their development have worn through their mitochondrial covering.

CELL-WALL.—The cell-wall of molds is quite distinct and often thick. It is sometimes cutinized. According to Mangin, it consists of callose and pectose with which is often associated a kind of cellulose.

SPECIFIC CONSIDERATION OF MOLDS*

A few species are found to grow very constantly in the same situations as bacteria. These are associated with forms of decay, fermentation, or disease, either as primary or secondary causes. They thus become important to the bacteriologist who studies them by the same methods as bacteria. These species belong to widely scattered groups of fungi, so that species found under the same conditions frequently differ greatly in appearance. The common term, molds, is applied collectively to these organisms, though no sharp limits can be set to the use of the term. Physiologically these species can be considered in three series:

COSMOPOLITAN SAPROPHYTES.—Certain species are capable of growing within very wide limits of temperature and of composition of substrata. Many of these have accompanied man everywhere and are

*Prepared by Charles Thom.

constantly found upon every kind of putrescible matter, especially as the causes of fermentation or decay in food. Their spores (conidia) are produced in countless numbers, and are so light that they float in air currents and are carried by contact in every conceivable manner by animals and by man. The life cycle from spore to spore is frequently very short, often being completed in twenty-four hours or less. Many of these forms are propagated for an indefinite number of generations by asexual spores or conidia, while for some of them no sexual-fruiting form is known. These species are the "weeds" of the bacterial culture-room, since they cannot be entirely eliminated and will survive, as a rule, conditions more severe than the bacteria themselves.

Molds of Fermentation.—A few species have acquired special importance by their fermentative action. In most cases these forms are widely distributed and able to utilize other media and conditions also. They differ from closely related species of the same genera in the ability to produce special enzymes or specially large amounts of such enzymes as bring about particular forms of fermentation. Certain of these species have been utilized in the manufacture of drinks, of citric acid, in cheese ripening, etc. Others are so adapted to growth under conditions of fermentation as to be found constantly in connection with such processes, in which their vigorous growth and fermenting power seriously interferes with control of results.

Parasites and Facultative Parasites.—A few molds are found as primary agents in causing diseases of man and animals. Some others enter as secondary infections, but become pathogenic after entrance. These comprise species of *Aspergillus* and *Penicillium* which produce disease in the external ear of man, *Aspergillus fumigatus*, a cause of lung disease of birds, and the series of forms causing skin diseases, dermatomycoses, of both man and animals.

Generic Consideration of Groups*

The Mucors or Black Molds.—The mucors or black molds constitute a large group of species belonging to the *Phycomycetes* or algal

*The series of forms presented contains representatives of the most common groups as they occur in laboratory cultures, and such as have acquired importance to the worker in bacteriology by participation in processes regularly studied by the bacteriologist. For more complete discussion of the fungi, the student is referred to standard text-books of cryptogamic botany. For discussions of species, Lafar's Technical Mycology includes the groups found associated with the bacteria; for other groups, special botanical literature must be consulted.

fungi whose general characters are a unicellar mycelium, at least in the vegetative stage, and quite generally a well-developed form of sexual reproduction (Figs. 31 and 32). In the mucors, the mycelium is usually richly developed within and often also on the surface of the substratum; asexual reproduction is accomplished by spores borne as conidia or borne within sporangia; and sexual reproduction is accomplished by the conjugation of special branches from the mycelium forming *zygospores* (Figs. 31 and 32). The typical mucors produce sporangia as capsule-like dilations at the ends of erect fertile hyphæ, each containing many spores. Septa are commonly developed in the mycelium when sporangia begin to appear. These fertile hyphæ may be microscopic or attain a length of several centimeters.

Important Species.—Perhaps the commonest form is *Rhizopus nigricans* (syn. *Mucor stolonifer*), the black mold of bread, a cosmopolitan species associated with the decay of many kinds of food stored in wet condition or in humid situations. Typical clusters of *sporangiophores* are borne on *stolons* or runners, which are hyphæ extending radially from the center of the colony and fastened to the substratum or to the support at intervals by root-like outgrowths. Abundant growth of this species is found only under very moist conditions or in substrata with high water content. *Rhizopus* is a very common contamination in laboratory cultures.

There are many common species of the genus *Mucor*, very few of which are identifiable without critical study. The specific names as commonly cited often designate groups of species or varieties rather than sharply marked forms. Certain of these may be briefly considered.

Mucor mucedo L. is a common form upon dung, characterized by heads (sporangia) upon long sporangiophores,* at first yellow then becoming dark brown or black and studded upon the surface with needles of lime.

Mucor racemosus, Fresenius, is characterized by the production of chlamydospores or cysts in the mycelium within the substratum, as elliptical thick-walled cells. The sporangiophores typically branch to make racemes of sporangia. The racemose mucors are active agents in

* The term *sporangiophore* is composed of the word *sporangium* combined with the suffix *phore*, meaning bearer. In sympodial branching the first fruit is on the tip of the original hypha, the first branch arises below this fruit and is terminated by the second fruit. Each successive branch and fruit originates in similar manner.

changing starch to sugar and in the production of traces at least of alcohol from sugars.

Mucor rouxii (Calm.), Wehmer, is the most important of a series of forms with sporangiophores branching sympodially which are active in changing starch to sugar and in producing traces at least of alcohol. The mycelium of *Mucor rouxii* develops in fluid cultures as yeast-like cells and groups of cells. The typical mucor fruits are produced only under special cultural conditions.

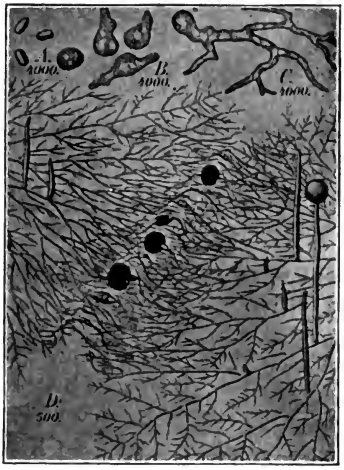


FIG. 31.—*Mucorineæ. Mucor. From Tabulæ Botanicæ*, showing sporangia originating from mycelium, spores and spore germination, and the formation of zygospores in a heterothallic species (diagrammatic). (Reduced one-half.) (*By permission of A. F. Blaskeslee.*)

Fermentation activity has been described for numerous species of *Mucor* and *Rhizopus*. Many of these species have been found and described as constituents of *Chinese yeasts*, or isolated in the study of the fermentation industries of Japan, China, and other eastern countries. Among them are *Mucor circinelloides*, Van Tieghem, *Mucor javanicus*, Wehmer, *Mucor plumbeus*, Bonorden, *Rhizopus oryzæ*, Went, *Rhizopus javanicus*. The fermenting power of mucors like that of yeasts varies greatly with the species or even with races used, approaching in some species the efficiency of the more active yeasts.

THAMNIDIUM.—Of related genera, *Thamnidium* differs from *Mucor* in the production of two kinds of sporangia. The terminal sporangium of a fruiting hypha resembles that of *Mucor;* the secondary or accessory sporangia which are borne upon side branches of the sporangiophores are smaller, lack the columella, and produce few to several spores within an outer wall.

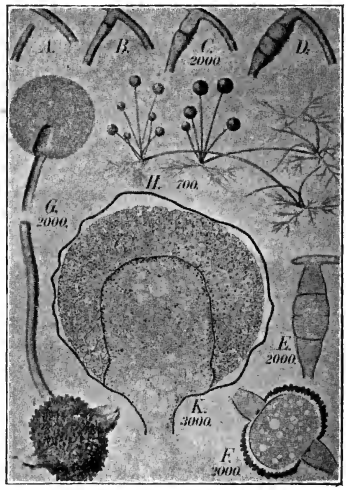


FIG. 32.—*Mucorineæ. Mucor, Rhizopus. A, B, C, D,* Formation of the zygospores from conjugating branches; *E,* section of *D; F,* mature zygospores in section; *G,* germination of zygospores; *H,* diagram of fruiting stolons of *Rhizopus nigricans; K,* section of sporangium during spore formation, highly magnified (*From Tabulæ Botanicæ.*) (Reduced one-half.) (*By permission of A. F. Blaskeslee.*)

Thamnidium elegans, Link, produces primary and secondary sporangia on different hyphæ, together making white colonies. The fertile side branches are produced in whorls and bear whorls of branchlets from their centers which in turn produce sporangioles from the tips of short straight twigs or branchlets.

PENICILLIUM.—The extremely abundant green molds most frequently belong to the genus *Penicillium,* although some members of other groups may be confused with them at times.

Characters.—Colonies are composed of loosely woven hyphæ, branched, septate, colorless, or bright colored. The fertile hyphæ

conidiophores) are mostly erect, arising either from submerged hyphæ, r as branches of aerial hyphæ, septate, usually branched only in the ruiting portion. Conidial fructifications consist of more or less com-

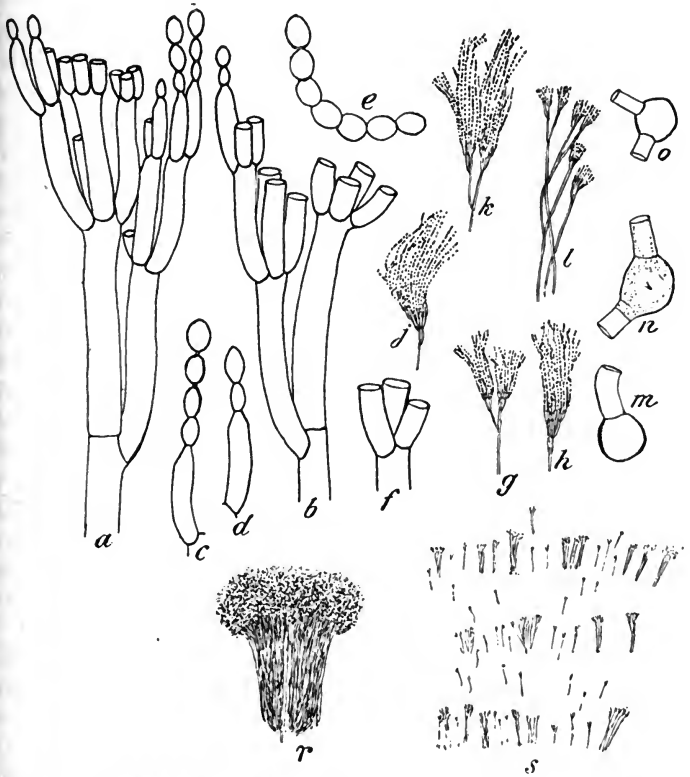


FIG. 33.—*Penicillium expansum*, Link. *a*, *b*, *f*, Branching and arrangement of branches of conidial fructification (×900); *c*, *d*, *e*, conidiiferous cells and conidial chains (×900); *g*, *h*, *j*, *k*, *l*, sketches of fructifications (×140); *m*, *n*, *o*, germination of conidia (×900); *r*, *s*, sketches from photographs showing in *s* loose aggregations of conidiphores beginning to develop into zonately arranged coremia, in *r* a coremium 1 mm. in height. (*From Bul.* 118, *Bureau of Animal Industry, U. S. Dept. Agriculture.*)

plex systems of branches and branchlets, the ultimate fertile cells each producing a chain of conidia (Fig. 33). The whole system is usually grouped near the end of the conidiophore, giving the appearance of

one or more brooms or brushes (whence the name). Very few species are known to produce asci, hence these are rarely encountered. The conidial form continues for an indefinite number of generations, therefore all the activities of the genus are associated with this form.

Cultural Considerations.—Among the numerous species and races, some of the green forms are widely distributed and almost omnivorous in habit. Other species are closely restricted to particular substrata. Starches and sugars appear to be especially favorable components of nutrient media for members of the group. The larger number of the species grows best at temperatures from 15° to 30°; a very few of them reach their optimum at 37°, but many species are entirely inhibited and some killed at blood-heat. Vegetative mycelium begins to be produced at temperatures very close to freezing, but colored conidia are produced slowly or not at all at low temperatures. The species of *Penicillium* thrive through a wide range of concentration of culture media, though perhaps the most characteristic growths are produced in media high in water content. The common species of each genus will grow in all the standard bacteriological media. With few exceptions the species grow well in synthetic media composed of assimilable carbohydrates and inorganic salts. A few species require the presence of some one of the higher nitrogenous compounds, but many species refuse to produce typically colored fruit without some form of starch or sugar in addition to ordinary peptone and beef-extract. Very few species grow well in alkaline media, but most species are tolerant of organic acids at the concentrations found in fruits and vegetables.

Some Common Species.—Penicillium roqueforti, Thom, is a green form constantly found in pure culture in Roquefort cheese, frequently also in ensilage. It is widely distributed and grows under many sets of conditions.

Penicillium camemberti, Thom, is the chief organic agent in ripening Camembert cheese. Cultures of this species are floccose or cottony, at first white, later gray-green.

Penicillium expansum, Link, is a green form, always obtainable from apples decaying in storage, upon which it frequently produces large *coremia.* It is one of the most abundant species of the genus, widely distributed in different countries. In cultures, colonies produce a characteristic odor, suggestive of its common habitat, decaying apples.

Penicillium brevicaule, Saccardo, is a form with rough or spiny brown

spores which has been used physiologically to detect the presence of arsenic by its ability to set free arsine from such substrata. Except species associated with particular processes or substrata, the identification of the green species of *Penicillium* requires special methods and greater care than is possible aside from special study of the group.

ASPERGILLUS (AND STERIGMATOCYSTIS).—The genus *Aspergillus* includes numerous species which develop under widely different conditions. Many of these forms reach their typical development under drier conditions than *Penicillium* and *Mucor*, such as stored grain, herbarium specimens, dried flesh, or foods containing concentrated sugars, such as jams, jellies, etc. Some excite processes of fermentation, and a few are associated with diseases.

Characters.—The vegetative hyphæ are creeping, submerged in the substratum or sometimes aerial also, loose, floccose, branched, septate, usually colorless, and sometimes bright colored. Conidiophores or fertile hyphæ are erect, unseptate, or few-septate, usually much larger in diameter than the vegetative hyphæ, and gradually enlarged upward, ending in a more or less abrupt dilation or head which bears closely packed columnar sterigmata or conidiiferous cells over the whole or a large part of its surface (Fig. 34, *b*). Each of these cells bears, in one group of species, a single chain of conidia, in other species (called by some authorities *Sterigmatocystis*) three or four secondary sterigmata which bear the conidial chains. Part of the species produce also thin-walled perithecia as yellow or brown spherical bodies upon the surface of the substrata. These perithecia are filled with eight-spored asci (Fig. 34, *e*). A few species produce sclerotia instead of perithecia, but many species are not known to produce either perithecia or sclerotia.

Important Species.—Among the species constantly met with, *Aspergillus niger* is recognizable by its black or very dark brown spores and in some strains by black sclerotia. Several black-spored forms are described, but their separation is usually impossible from the data given. *Aspergillus niger* ferments sugar solutions with the production of oxalic acid in considerable quantity.

Of green forms, *Aspergillus** *glaucus*, Link (*Aspergillus herbariorum*, Wiggers), and *Aspergillus repens*, De Bary, both produce abundant yellow perithecia. These abound upon herbarium specimens, hay,

*Recent examination of a large number of American specimens shows that *Aspergillus repens* is the usual green form in this country.

grain, concentrated foods, such as jellies, preserves, and dried meats upon which they produce green conidial areas which are later dotted with bright yellow perithecia.

Aspergillus fumigatus, Fresenius, is a green form characterized by short conidiophores enlarging gradually into heads and bearing a single set of sterigmata on the very apex, with chains of thin-walled green spores about 3μ* in diameter. This species produces a destructive disease of birds known as aspergillosis. The same species is sometimes reported as pathogenic to man.

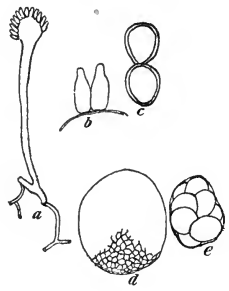


FIG. 34.

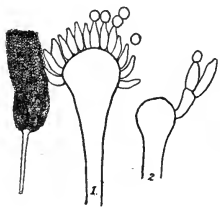


FIG. 35.

FIG. 34.—*Aspergillus glaucus*. *a*, Conidiophore showing increased diameter over the vegetative cells at its base (×128); *b*, sterigmata (×450); *c*, conidia, smooth thick walled in this variety, other varieties are spiny (×450); *d*, perithecium(×128); *e*, ascus containing ascospores (×450). (*Original*)

FIG. 35.—*Aspergillus*. (1) *A. fumigatus*, Fres; (2) *A. nidulans*. 1 and 2 show the simple sterigmata of *A. fumigatus* and the secondary sterigmata of *A. nidulans*. The conidia of these species do not remain attached in ordinary fluid mounts. (*Original.*)

Aspergillus nidulans differs by having two sets of sterigmata, but otherwise frequently closely resembles *Aspergillus fumigatus* and is frequently mentioned as pathogenic.

Aspergillus oryzæ has been used to produce "Taka-diastase" from rice in Japan. Other species produce amylase also, but in different degrees.

Aspergillus wentii, Wehmer, characterized by its long conidiophores and coffee-colored heads of conidia, is found in the soja preparation in Java.

Of other forms constantly met, *Aspergillus candidus* has white or

*The unit of measurement is the *micron* (μ) or *micro-millimeter* (.001 mm.or $\frac{1}{25000}$ in.)

pale cream fruiting surfaces, *Aspergillus flavus* produces several shades of yellow and green, *Aspergillus ochraceus*, ocher or tan.

Much confusion is still found in the literature of this genus, so that frequent references to the activities of particular species are difficult or impossible to verify.

CLADOSPORIUM (AND HORMODENDRON).—The species of *Cladosporium* occur frequently in cultures of decaying vegetable matter, of milk and cream, or butter. The colonies liquefy gelatin. Both mycelium and spores are at first colorless, but later dark colored to almost black, with spores becoming two-celled in very old cultures.

Cladosporium herbarum is the commonest species encountered.*

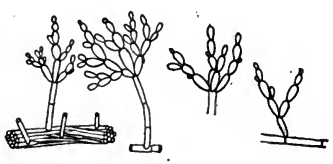


FIG. 36.

FIG. 37.

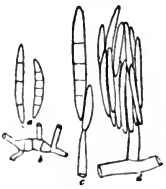


FIG. 38.

FIG. 36.—*Cladosporium herbarum*, showing the forms of conidiophores and conidia which are very common upon laboratory culture media. (*Original.*)

FIG. 37.—Spores of *Alternaria sp.* (*Original.*)

FIG. 38.—*Fusarium* from decaying potato. *a*, Spores showing curvature and septa; *b*, germination of spores; *c*, development of spores in petri-dish culture; *d*, mass of spores as found in culture. (*Original.*)

Colonies in culture media differ so greatly in structure from those upon natural substrata as to make identification of species questionable. Fig. 6. Much confusion is therefore found in the use of the names of species of *Cladosporium* and the related genus, *Hormodendron*, which is separated by some.

ALTERNARIA AND FUSARIUM.—The frequent occurrence of species of *Alternaria* and *Fusarium* in cultures demands that the generic characters be recognized. Both, as a rule, produce abundant growth with a tendency to over-run cultures of other forms (Figs. 37 and 38). The spores of *Alternaria* are brown, Indian-club form, muriform (divided into several cells by longitudinal as well as cross-walls), and are connected together into chains (Fig. 37). The spores of *Fusarium* are

* This species has been shown to be a conidial form of *Sphærella tulasnei* Janczewski, but the bacteriological student will meet only the conidial stage.

colorless, either straight or sickle- or crescent-shaped, divided into several cells by cross-walls occur singly or adhere in masses on the tips of the fertile branchlets. The morphology of colonies in culture varies widely from the descriptions of the same species under natural conditions. Species of *Fusarium* frequently produce bright colors in the mycelium and substrata; colonies of *Alternaria* often become almost black. Identification of species in cultures is thus far impossible, except for the specialist.

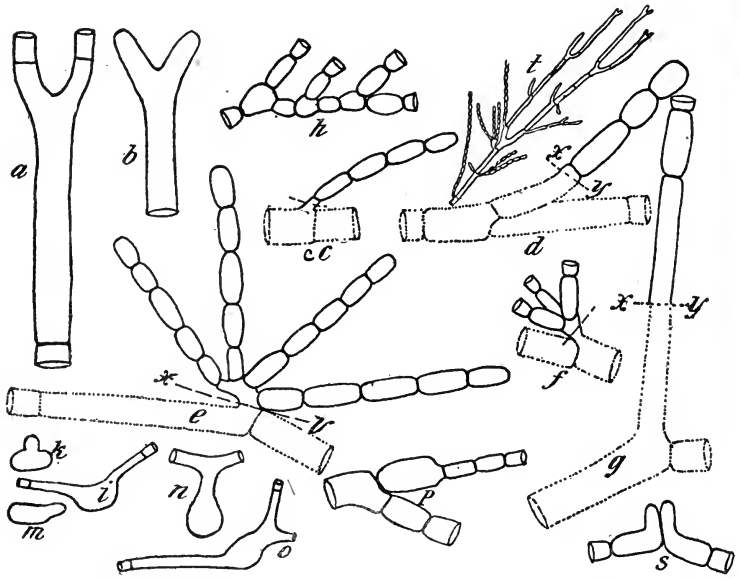


FIG. 39.—*Oidium lactis*. *a, b,* Dichotomous branching of growing hyphæ; *c, d, g,* simple chains of oidia breaking through substratum at dotted line *x–y*, dotted portions submerged; *e, f,* chains of oidia from a branching out-growth of a submerged cell; *h,* branching chain of oidia; *k, l, m, n, o, p, s,* types of germination of oidia under varying conditions; *t,* diagram of a portion of a colony showing habit of *Oidium lactis* as seen in culture media. (*From Bull.* 82, *Bur. Animal Industry, U. S. Dept. Agr.*)

OIDIUM.—*Oidium* (*Oospora*) *lactis* is universally found in cultures from milk and milk-products and very frequently in decaying vegetables, manure, etc. Colonies of the species are colorless, have vegetative mycelium entirely submerged, become powdery white with

spores when mature, liquefy gelatin, and produce a strong characteristic odor (Fig. 39). Microscopically the species is recognized by dichotomous branching of the hyphæ at the margin of the colonies, and by the spores or oidia which are abruptly cylindrical, varying with conditions in length and diameter and produced both above and below the surface of the substratum in long chains which break up readily. At times the whole mycelium appears to break up into oidia. *Oidium lactis* is a factor in the ripening of many kinds of cheese: Limburg, Harz, Camembert, Gorgonzola, etc. Its activity is associated with strong odor and taste.

Monilia.—*Monilia candida* (Bonorden), Hansen. The line between the *Mycoderma* group of yeasts, *Oidium* and *Monilia*, and the

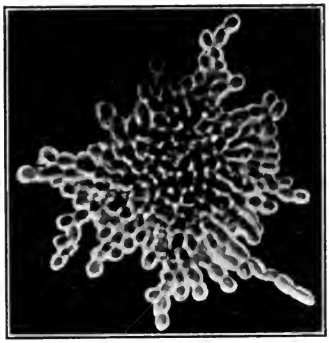


Fig. 40.—A colony of *Monilia candida*. (*Photographed by Z. Northrup.*)

well-fixed mold types shows a number of organisms which are found repeatedly in the fermentation industries (Fig. 40). One of these, *Monilia candida*, as described by Hansen, has been much studied. In morphology, *Monilia candida* appears as a yeast in young cultures in sugary fluids, but later develops a mycelium. It produces an alcoholic fermentation which increases in vigor with the rise of temperature toward 40°.

Dematium.—One species of *Dematium*, *Dematium pullulans*, has been much studied. This is frequently found within decaying fruit as dark brown colonies. In culture, mycelium is sparingly produced, either colorless or colored, and conidia are borne in clusters and chains

all along the hyphæ submerged in the substratum. At first both mycelium and conidia are colorless, later some or all of the cells develop heavy dark brown walls. Although not active as an agent of fermentation, it occurs very frequently in the fermentation industries sometimes discoloring the fermenting products. The conidia bud out from the cells of the mycelium in a manner resembling the yeasts. Its occurrence with the yeasts has led to many careful descriptions of its several types of spore production and its biological activities.

SAPROLEGNIACEÆ AND ENTOMOPHTHORACEÆ.—These are two groups of *Phycomycetes* which differ from the mucors in habit and in their prominent development of sexual reproduction.

ENTOMOGENOUS FUNGI.—Numerous species have been identified as the destroyers of particular insects.

CHAPTER III

YEASTS*

Morphology of Certain Types

Definition and Bases of Classification.—If the cloudy freshly expressed juice of grapes or other fruits be passed through a centrifuge, the sediment will be found to consist principally of amorphous particles of dirt and plant tissue. If the clear juice is now allowed to stand in a warm place for a few days it will ferment and the sediment thrown down by the centrifuge may be shown by the microscope to consist principally of unicellular microörganisms.

These microscopic cells are called collectively "yeast" and belong to various groups of fungi. Some of them are special vegetative forms of *Phycomycetes* (*Mucor*), others of *Ascomycetes* (*Saccharomyces*, *Aspergillus*), while others are unknown in any other form and are classed as *Fungi imperfecti* (*Mycoderma*, *Torula*). They are widely distributed in nature and some of them occur on all exposed surfaces and particularly on moist organic substances containing sugar and acid. The true yeasts (*Saccharomycetes*), which are of the greatest importance industrially, occur naturally on the raw material (*S. ellipsoideus* on grapes) or are known best in the cultivated condition (*S. cerevisiæ* of beer).

The true yeasts occur in the form of spherical or more or less elongated cells varying in normal width from 2.5μ to 12μ. The first classifications were based on shape and size alone but these vary and depend so much on cultural conditions that they are of little value in differentiating species or varieties.

The range of variation in shape and size, especially of the spores, under given conditions of culture medium and temperature, is now used only in conjunction with the reactions brought about in various solutions to distinguish the various forms.

The true yeasts are characterized by the formation of endospores and are classed with the *Gymnoascæ*. Each cell seems capable, under

* Prepared by F. T. Bioletti. A. Guilliermond has furnished the section on the "Cytology of Yeasts."

favorable conditions, of developing into an ascus. Many unsuccessful attempts have been made to connect the true yeasts genetically with various forms of fungi such as *Mucor*, *Ustilago* and *Dematium*. At present they must be considered as distinct species.

Some yeasts have a tendency during fermentation to remain at the bottom of the liquid; others form a thick foamy layer on top. These are known respectively as *bottom* and *top* yeasts. No sharp distinction can be made as there are intermediate forms.

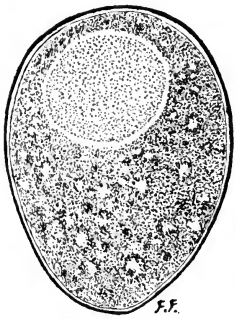


FIG. 41.—Yeast cell. (*Original.*)

The vegetative reproduction in the genus *Saccharomyces* takes place by budding, in *Schizosaccharomyces* by fission.

The extreme temperatures for budding lie between 1° and 47°, varying with different species. The optimum temperature varies in the same way between 25° and 35°. The rate of multiplication under favorable conditions will range from one to several hours for the formation of a new cell.

When young, vigorous, well-nourished cells are supplied with abundant air and moisture at a comparatively high temperature under conditions that discourage budding (lack of nutriment) they form *endospores*. These spores are usually about half the diameter of the mother cell and from one to eight or more may occur in each cell. They may be formed by cells before or after budding and may even change to asci and form new spores. They are generally spherical or slightly ellipsoidal, rarely kidney-shaped (*S. marxianus*) or furnished with a zonal ring (*S. anomalus*) (Fig. 42).

In nutrient solutions they swell, burst the mother cell, become free and germinate by budding, usually producing vegetative cells directly, though occasionally producing first a short promycelium (*S. ludwigii*).

In *Schizosaccharomyces octosporus* the ascus is formed by the fusion of two cells. Sometimes in other species, two or more spores in one cell will fuse before germination.

Staining with warm carbol-fuchsin and partial decolorization with weak acetic acid leaves the spores red and the cell colorless.

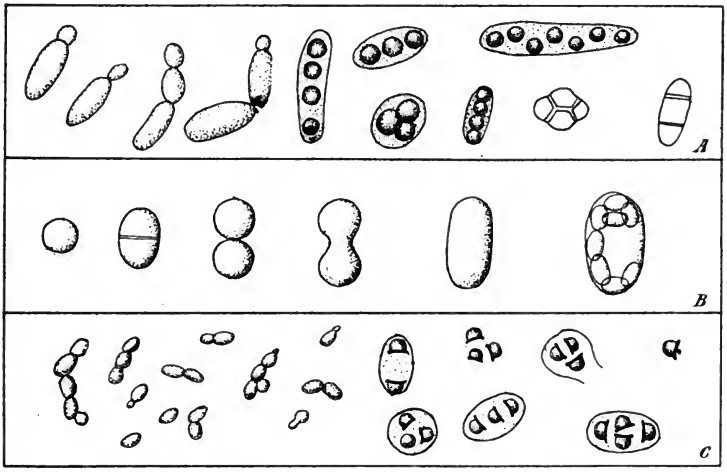


FIG. 42.—Spore-bearing cells. *A. S. pasteurianus.* (*After Biolctti.*) *B. Sch. octosporus.* (*After Schiönning.*) *C. S. anomalus.* (*After Kayser.*)

CYTOLOGY OF YEASTS*

GENERAL STRUCTURE OF YEASTS.—The structure of yeasts in no way differs from that of the other fungi, only it is seemingly more complex and consequently more difficult to interpret on account of the abundance of the stainable granulations which sometimes accumulate in the cells and occasionally hinder the differentiation of the nucleus. This explains why it has until recently remained a subject of controversy. It is now fairly well understood.

* Prepared by A. Guilliermond.

In order to understand clearly this structure, one must observe young cells taken from a culture at the beginning of development. For this purpose we use *Saccharomyces cerevisiæ* which, because of the relatively large size of its cells, lends itself better than any other yeast to a cytological study. Examined in the living state, highly magnified, the cells of this yeast show a dense and homogeneous cytoplasm with a group of small vacuoles or a single large vacuole at the center. In the vacuoles and also in the perivacuolar cytoplasm, we can clearly distinguish a great many small shining granules, of varying sizes, which manifest Brownian motion. It is easy to stain them in the living state (Fig. 43) with a very dilute solution of neutral red or methylene blue. These are only metachromatic corpuscles.

FIG. 43.—*Saccharomyces cerevisiæ.* Young cells examined in the living state in a solution of neutral red. The vacuoles, stained pale red, contain metachromatic corpuscles colored dark red.

In fixed and stained preparations (Fig. 44, 1–10) is seen in each cell a single, large nucleus, whose structure is exactly like that which we have discussed in molds. This nucleus is surrounded by a membrane and contains a hyaline nucleo-

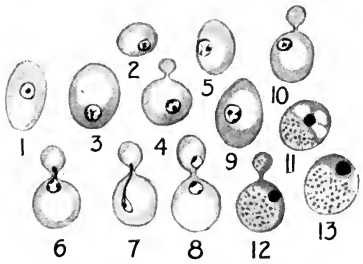


FIG. 44.

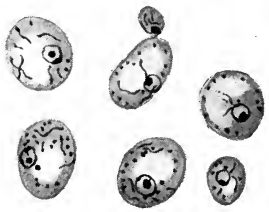


FIG. 45.

FIG. 44.—*Saccharomyces cerevisiæ.* 1–10, Young cells with nucleus, showing its structure. 6–8, The same: division of the nucleus. 11–13, Cells after twenty-four hours' fermentation, with a very large glycogenic vacuole filled with lightly colored grains.

FIG. 45.—*Saccharomyces cerevisiæ.* Young cells fixed and stained by a special method revealing in the cytoplasm a chondrium consisting of rod mitochondria and granular mitochondria.

plasm in which is easily seen a large nucleolus and some chromatin; this latter is scattered through the nucleus, sometimes found in the nucleoplasm in the form of a network, sometimes reduced to a num-

ber of granules smaller than the nucleolus, and sometimes even found gathered on the circumference of the nuclear membrane.

The cytoplasm is dense and homogeneous. A special technic has recently enabled the demonstration of a chondrium in the cytoplasm. This seems to consist both of granular mitochondria and of more or less elongated and flexible rod mitochondria (Fig. 45).

The vacuole shows in its interior numerous metachromatic corpuscles of varying sizes (Fig. 46). As in molds, these corpuscles appear not only in the vacuole, but also in the perivacuolar cytoplasm; there they start, and are next diffused in the vacuole where they finish their growth, then dissolve when the need is felt. It is difficult in the case of yeasts to determine their origin; nevertheless, observations made of fungi with larger cells than we have previously described, show that the metachromatic corpuscles start in the midst of mitochondrial elements, and it seems certain that after that the process is the same in yeasts.

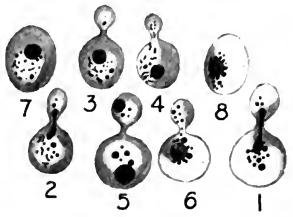


FIG. 46.—*Saccharomyces cerevisiæ*, stained by a method revealing both the nucleus and the metachromatic corpuscles.

In the cytoplasm of yeasts, also, have been noted granulations, which can be stained with ferric hæmatoxylin, which have been named *basophile grains;* but these formations, which are not well defined, seem to us to represent simply products from the alteration of the chondrium under the influence of imperfect fixing agents.

The membrane of yeasts is quite thick and very distinct. Its chemical nature is still little known. According to some authors, it consists of a cellulose; others think that it contains only pectose. According to Mangin, it is formed of callose. Finally, some authors have thought they discerned chitin.

The structure we have just described is found in all the species (Fig. 47), only it is sometimes much less distinct because of the smallness of the cells. In the elongated yeasts, and in the cells composing the mycelial formation which are encountered under some conditions, especially in the films, the nucleus generally occupies the center of the cell; it is situated in a kind of matrix or bridge consisting of a very dense cytoplasm, while a vacuole filled with metachromatic corpuscles occupies each of the two extremities of the cell.

Summing up the elements of which a yeast cell consists are a cytoplasm with a chondrium, a nucleus with clearly differentiated structure, vacuoles containing numerous metachromatic corpuscles, a membrane of a nature not yet clearly defined.

Cytological Phenomena during Multiplication.—During the budding of the yeasts, cytoplasm enters the young bud with some chondrium; then, when the bud has reached a certain size, the cytoplasm forms in it a little vacuole in which appear metachromatic corpuscles (Fig. 46, 2–7).

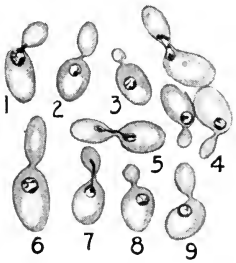


Fig. 47.—*Saccharomyces ellipsoideus*. Young cells with nucleus.

In the course of these phenomena, the nucleus retains the position which it occupied in the mother cell before the appearance of the bud. Only when the bud is quite large does the nucleus begin to divide. It is elongated so that one end penetrates the bud; the nucleus then resembles an elongated dumb-bell with the larger head remaining in the mother cell and the other, smaller head, in the bud (Fig. 44, 6, 7 and 8; Fig. 46, 2, 7; Fig. 48). Soon the part of the dumb-bell which is stretched out breaks near the neck of the bud, forming two nuclei of unequal size, at first tapering spherical in shape, and later rounded off: one is the nucleus of the mother cell and the other that of the bud. This division is therefore effected by the direct method; it is an *amitosis*. In the *Schizosaccharomyces*, where the cells do not multiply by budding as in other yeasts, but by a transverse partition, the nuclear division is effected by amitosis: the nucleus, situated in the center of the cell, elongates along the longitudinal axis of the cell and resembles a dumb-bell, ending by dividing in the middle, thus forming two nuclei of the same size. Soon a transverse septum appears between the two nuclei and separates the two daughter cells.

We have now to note the modifications which arise in the structure of the cells during the different phases of development and at the time of sporulation.

Variation in the Cellular Structure during Development.—In the course of development, especially during fermentation, yeasts reveal cytological phenomena which render their structure more complex and more difficult to interpret. Let us take for example the study

of the *S. cerevisiæ*. After twelve hours of fermentation, the metachromatic corpuscles become more numerous. At the same time, the cytoplasm forms little vacuoles which contain no metachromatic corpuscles, but only glycogen, easily detected by iodo-iodide of potassium. These are gradually fused into a single vacuole, which enlarges much and modifies materially the cell structure. The glycogenic vacuole, increasing, pushes back to the periphery of the cell the cytoplasm, the vacuoles with metachromatic corpuscles, and the nucleus whose chromaticity increases and which becomes homogeneous in appearance (Fig. 44, 11). After forty-eight hours, moreover, the cell is found to consist of an enormous vacuole filled with glycogen which occupies most of it, while the nucleus, the vacuoles with metachromatic corpuscles and the cytoplasm are pushed back to one side of the cell, which is then transformed into a kind of glycogen sack (Fig. 44, 12 and 13; 46, 6–8). At this time the glycogenic vacuole contains a great many small granulations (Fig. 44, 12–13), which easily fix some staining materials, especially ferric hematoxylin, and whose origin and significance have not been determined.

Toward the end of fermentation, the glycogen gradually diminishes and the glycogenic vacuole is gradually reduced, then ends by disappearing. The cell after this resumes its original structure.

In the course of these phenomena, the membrane apparently shows no modification. It is known, however, that under some conditions, yeasts secrete gelatinous substances which englobe their cells in a kind of jelly and so appear like zoöglœa (Hansen). It is well to add, on the other hand, that many pathogenic yeasts, when living in the host, have the ability to protect their cells against the reaction of the organisms, by secreting a very thick capsule of gelatinous nature: each of their cells is then surrounded by a large capsule.

Cytological Phenomena of the Sporulation and Germination of Ascospores.—For a study of the sporulation, we will consider a representative of the species *Schizosaccharomyces*, the *Sch. octosporus*, in which these phenomena are easily observed and especially well understood.

We know that in this yeast, as in some others, sporulation is preceded by a sexual phenomenon consisting of an isogamous copulation. The ascus results from the fusion of two similar cells. The gametes are ordinary cells which have the structure which we have previously

described, with one nucleus and one or more metachromatic vacuoles containing corpuscles (Fig. 48, *a*). Fusion takes place between the two cells which are nearest together. Each of these two cells sends out a tiny beak; the two little beaks thus formed anastomose and form a channel of copulation joining the two cells (Fig. 48, *b*, *c*, *d*). The septum separating the two gametes in the middle of the channel is quickly absorbed, and the two cells then have free communication. The cytoplasm of the two cells draws together and mingles in the channel; there the two nuclei draw near to each other (Fig. 48, *e*) and fuse into a single nucleus (Fig. 48, *f*, *g*, *h*). Next the zygote ends its fusion; instead of its original dumb-bell appearance, it assumes the form of an oval cell, then grows large (Fig. 48, *i*). Occasionally, however, it retains a vestige of the individuality of the two gametes, showing two swellings joined by a somewhat narrower middle portion (Fig. 48, *j*).

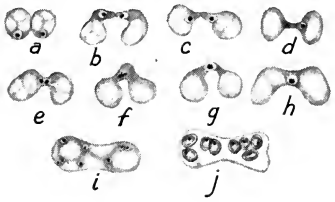


FIG. 48.—Successive stages of copulation and sporulation in *Schizosaccharomyces octosporus*.

During this time, the cell becomes filled with little vacuoles and assumes a more or less alveolar structure. These vacuoles contain a number of metachromatic corpuscles. The nucleus which occupies the center of the zygote begins to divide. The ascus, containing sometimes four, sometimes eight ascospores (Fig. 48, *j*), will then undergo two or three successive divisions, as the case may be. These divisions are accomplished by *karyokinesis* or *mitosis*. In the stages preceding nuclear division, the nucleus is very large and shows a very clear structure with a nucleolus and a chromatic reticulum (Fig. 49, *a*). It soon elongates and assumes a special structure. Its membrane loses its clearness, and in the midst of the nucleoplasm an achromatic spindle appears, ending at each of its two poles in a very small centrosome and containing at its center a group of fine granulations representing the equatorial plate (Fig. 49, *b* and *c*). The

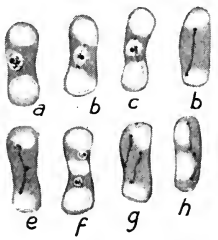


FIG. 49.—*Schizosaccharomyces octosporus*. Various stages of the nuclear division during sporulation.

nucleolus always persists on one side of the spindle. At a subsequent stage the chromatic granulations or chromosomes are divided between the two poles of the spindle, the nucleoplasm is mixed with cytoplasm, then the spindle elongates, while the chromatic granulations form a homogeneous mass at the two poles (Fig. 49 *d*, *e*, *g* and *h*). The nucleolus is quickly absorbed, then the two nuclei are formed at the expense of the two chromatic masses (Fig. 49, *f*). To summarize, therefore, this division consists in mesomitoses of a primitive kind, which appear to take place in the interior of the nucleus, whose membrane is absorbed only at the end of the phenomenon. They show the characteristics of the mesomitoses which have been described in the asci of the higher Ascomycetes.

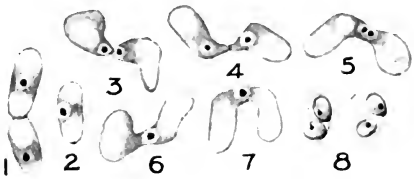


FIG. 50.—Successive stages of copulation and sporulation in *Schizosaccharomyces pombe*. 1–2, Cells just as sporulation is about to begin. 3–7, Union of the two gametes and nuclear fusion. 8, Ripe ascus. Cellular fusion being incomplete, the ascus retains the shape of the two cells joined by a channel of copulation.

When these divisions are accomplished, the nuclei seem to be scattered in the cell (Fig. 48, *i*); they are soon surrounded by a thin layer of cytoplasm which is separated from the cytoplasm by a membrane; these are the ascospores. At first very small, these gradually increase at the expense of the cytoplasm which has not been used in their formation—in other words *epiplasm*—then reach the point where they occupy the whole of the ascus, after having absorbed this epiplasm (Fig. 48, *j*.) The metachromatic corpuscles scattered in the vacuoles of the epiplasm disappear during these phenomena, being absorbed by the ascospores. At no time during the development of the ascus can glycogen be seen any more than in plant cells, but this is replaced by an amyloid substance which is stained blue by iodo-iodide of potassium. This substance impregnates the membrane of the ascospores and disappears during their germination, utilized as a reserve product.

In some *Schizosaccharomyces* or ordinary yeasts which bud (zygo-

saccharomyces) the ascus comes from an egg which starts in a similar manner (Fig. 50.) In some species, this egg is formed by a heterogamous copulation between an adult cell (macrogamete) and a very young cell which has just separated from the mother cell (microgamete) (Fig. 51). On the contrary, in most species, the ascus results from the simple transformation of an ordinary cell without previous copulation. Whatever may be its origin, the ascus shows cytological phenomena quite similar to those which have just been described in *Sch. octosporus*, with mere differences of detail. Always in *Sch.*

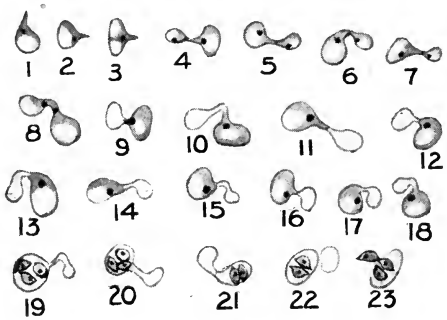


FIG. 51.—Heterogamous copulation in *Zygosaccharomyces chevalieri.* 1–3, Gametes sending out a beak in anticipation of copulation. 4–7, Micro- and macrogametes joined by their channel of copulation. 8, The partition separating the two gametes is absorbed. 9–18, The contents (nucleus and cytoplasm) of the microgamete enter the macrogamete and are fused with the contents of the latter. 19–21, Ripe asci. 22–23, Freeing of the ascospores by rupture of the membrane of the ascus.

octosporus are seen only a few metachromatic corpuscles in the ascus. In most of the other yeasts, on the contrary, the ascus contains a very large number of metachromatic corpuscles, and it is easier there to follow the evolution of these bodies which present interesting singularities clearly demonstrating their rôle as reserve substances.

Let us observe, for example, the cytological phenomena which appear during sporulation in *Saccharomyces ludwigii.* In this yeast, which shows no sexuality in the origin of the ascus, the cells which are preparing to sporulate assume a finely vacuolar structure (Fig. 52, 8 and 9) and produce a large quantity of reserve products: metachromatic corpuscles, glycogen and fat globules. Metachromatic corpuscles spring up in some vacuoles, glycogen in others; as for the fat globules, they

are located in the cytoplasmic web. The nucleus is situated on one side of the cell, surrounded by a thin layer of very thick and homogeneous cytoplasm which is to become the *sporoplasm*, at whose expense the ascospores are formed, the remainder—that is to say the vacuolar cytoplasm—being destined to compose the epiplasm or nourishing plasm.

At a later stage, the metachromatic corpuscles undergo a kind of pulverization transforming them into small grains, and begin to dis-

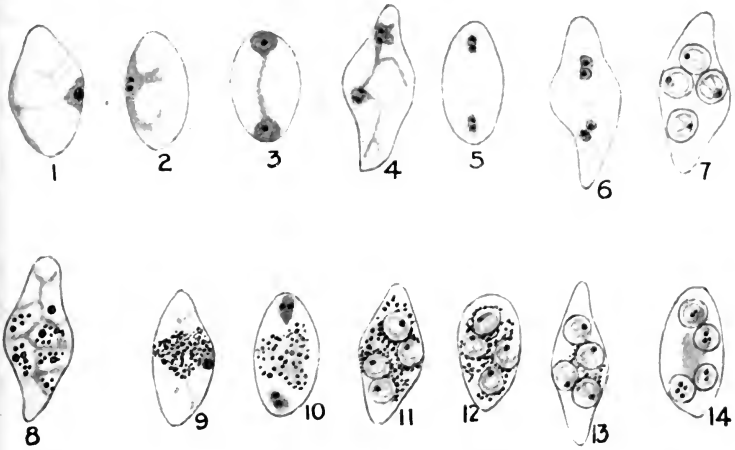


FIG. 52.—Sporulation in *Saccharomyces ludwigii*. Figs. 1 and 7 showing the evolution of the nucleus. Figs. 8–9, the metachromatic corpuscles, stained by a method permitting a differentiation, except in Fig. 8, are dissolving, and the substance of the vacuole which contains them shows a diffuse metachromatic coloring (here gray) like the corpuscles.

solve in the vacuoles surrounding them, the latter at this time taking, with aniline blue stains, a diffuse red coloring similar to that of the metachromatic corpuscles (Fig. 52, 9). At the same time, the nucleus undergoes two successive divisions, but these have not been discernible up to the present time, because of the density and the strong chromaticity of the sporoplasm surrounding the nucleus. They are manifested merely by the appearance of the two daughter cells which migrate to the two poles of the cell, carrying with them a part of the sporoplasm, which assumes the appearance of a dumb-bell and whose

slender part ends by breaking (Fig. 52, 2, 3 and 4). The cell, therefore contains at this time at each of its poles a small mass of sporoplasm having first one, then two, nuclei (Fig. 52, 5 and 10). After this, the sporoplasm condenses around each of these nuclei (Fig. 52, 6), thus delimiting at each of the poles two small ascospores.

During these phenomena, the metachromatic corpuscles congregate around the ascospores (Fig. 52, 11 and 12), then gradually dissolve. The ascospores constantly increase in size at the expense of the epiplasm, which becomes disorganized and is reduced to a vacuolar liquid containing in suspension metachromatic corpuscles, fat globules and glycogen. They succeed in absorbing entirely the epiplasm and in occupying the whole of the ascus (Fig. 52, 13 and 14). The metachromatic corpuscles, like the glycogen and the globules of fat, are then completely absorbed by the ascospores, which indicates clearly that they, as well as the latter substances, act as reserve products. When the ascospores are ripe, they contain in their vacuoles metachromatic corpuscles (Fig. 52, 14).

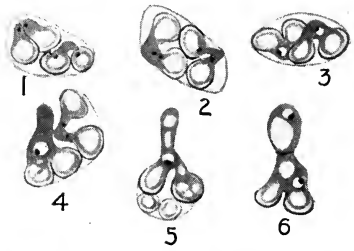


FIG. 53.—Germination of ascospores in *Saccharomyces ludwigii*. 1, Beginning of the fusion of the ascospores. 2, The ascospores are joined two by two by a channel of copulation, but their nuclei are not yet fused. 3, The nuclei are fused. 4, At the left two ascospores, joined, have formed at the middle of the channel of copulation a bud which has ruptured the membrane of the ascus. At the right, the two ascospores, joined by a channel of copulation have not yet fused their nuclei. 5, Formation of the bud at the expense of the two fused ascospores. Two other ascospores have not yet begun their fusion. 6, The bud formed at the channel of copulation is already established and separated from this channel by a transverse septum.

In all yeasts, at the time of budding, the ascospores have the appearance and structure of plant cells. Their germination does not differ from ordinary plant multiplication. In some species, however, especially in *S. ludwigii*, copulation, suppressed at the beginning of sporula-

tion, is replaced by a compensating phenomenon which intervenes at the germination and consists in the fusion of the ascospores two by two (Fig. 53). The ascospores anastomose at their extremities by a channel of copulation which, as soon as the nuclear fusion is accomplished, becomes the seat of a budding.

The Principal Yeasts of Importance to Fermentation Industries

True Yeasts, Saccharomycetes.—The various yeasts used in brewing and some of those used in producing distilling material are grouped together as *S. cerevisiæ*. They are large and round or slightly oval.

They are divided into three main groups—the *bottom yeasts* which are used in the manufacture of German beer, and which, usually, are capable of producing only a moderate amount of alcohol; the *top yeasts*, used in English beers and compressed yeast, capable of producing more alcohol, and the *distillery yeasts*, which have great fermentative power and produce large amounts of alcohol.

Many forms of these yeasts have been described in great detail by Hansen and others but the distinctions are based principally on physiological peculiarities such as the temperature and time limits of film and spore formation, and the character of the fermented liquids. The various forms seem to be fixed, and to retain their characteristics unchanged under almost all forms of treatment.

The wine yeasts, *S. ellipsoideus*, seem to be even more diverse than the beer yeasts, but have been less thoroughly studied. They are somewhat smaller than the latter and usually slightly more elongated. They form spores much more abundantly and easily than the beer yeasts and the cells in film formation are often much elongated.

Their fermentative power is considerable, some of them being capable of producing over 16 per cent by volume of alcohol. W. V. Cruess has obtained 21 per cent from a Burgundy wine yeast. They differ in the flavors and aromas which they produce in the fermented liquid, and especially in the rapidity with which they settle. Some yeasts, such as those of Champagne and Burgundy, form a compact sediment which settles quickly and leaves the liquid clear. Others remain suspended for a long time and settle with difficulty.

Every region seems to have its own forms and the characteristics of the various forms seem to be as well fixed as those of beer yeasts.

Wines are manufactured by the use of these yeasts. They are also employed in distilleries. In breweries they are considered *disease yeasts* and have a deleterious effect on the beer.

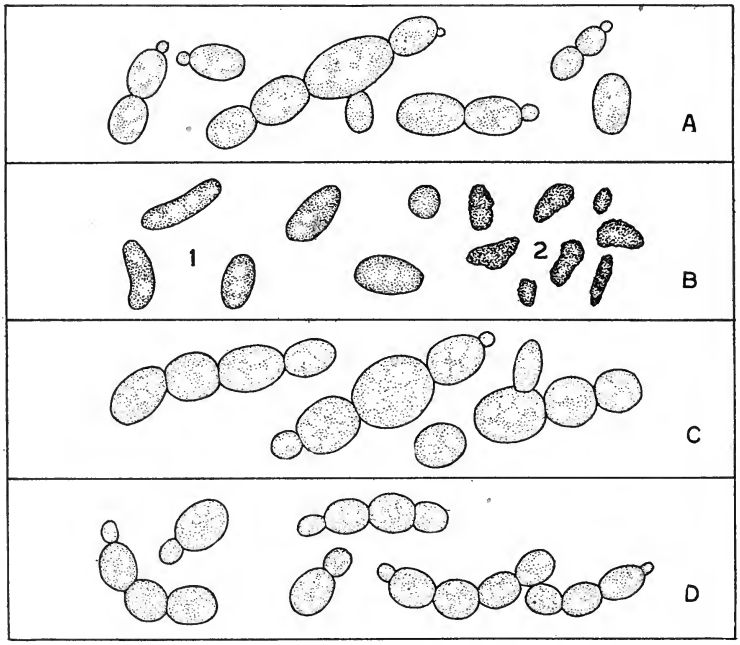


FIG. 54.—Wine and beer yeasts. *A*, *S. ellipsoideus*, young and vigorous; *B*, *S. ellipsoideus*, (1) old, (2) dead; *C*, *S. cerevisiæ*, bottom yeast; *D*, *S. cerevisiæ*, top yeast. (*Original.*)

S. pyriformis resembles in shape *S. ellipsoideus*, and in association with *Bacterium vermiforme* produces ginger beer.

S. vordermanni is concerned in the manufacture of *arrack*. It ferments the sugar produced from rice by the molds, *Mucor oryzæ* and *Rhizopus oryzæ*.

S. fragilis and other yeasts have been found in kefir and other fermented drinks made from milk. These yeasts working in conjunction with bacteria produce alcoholic acid beverages.

Many true yeasts are more or less injurious. They do not, like bacteria and pseudo-yeasts, cause serious diseases, capable of completely ruining the fermented product, but they may injure the quality more or less. Some yeasts are useful in certain cases and injurious in others. If beer yeasts become contaminated with wine yeast the resulting beer may be persistently turbid. If one attempts to ferment grapes with beer yeast, a wine with a disagreeable beer aroma and of poor keeping qualities is produced.

S. pasteurianus occurs in several forms as an injurious yeast in breweries, causing bitterness and turbidity. Similar forms occur in wine but do little harm except in the absence of the true wine yeast. The cells of this species vary from oval to long ellipsoidal, often being much elongated and in film formation sometimes producing a branching mycelium. Spores are formed easily and abundantly.

The apiculate yeast, *S. apiculatus*, is very abundant on grapes and most acid fruits. It is very variable and undoubtedly includes many varieties. The cells are small, vary in shape from oval to cylindrical, most of them having an apiculation at one or both ends, making them pear or lemon shaped. According to Lindner they form spores in drop cultures, one in a cell. Under favorable conditions this yeast increases with great rapidity, but is checked by 3 to 5 per cent of alcohol. It causes cloudiness in wine, interferes with the growth of the proper yeast and injures the flavor.

Many yeasts, mostly small and some of them rose-colored, have been found on grapes and in wine, but they do not develop under ordinary conditions of wine making sufficiently to be harmful.

Schizosaccharomyces pombe is a yeast found in *pombe* or *millet* beer, made by negroes in Africa. It is cylindrical and large, though variable in size. Both ends are rounded. It multiplies by forming a septum near one end, the smaller division then growing into a normal cell. From one to four spores are formed in a cell. These spores are often produced in the fermenting liquid. The fermentative power is high and a large percentage of alcohol may be formed.

Several other species of this genus have been isolated from grapes and from Jamaica rum.

Pseudo Yeasts.—Budding cells often occur in fermenting liquids which have all the characteristics of yeast except that of producing endospores. They are grouped together under the name of *Torula*.

They are usually small, spherical or slightly elongated. Some species produce a little alcohol and some none. They seldom occur in sufficient quantities to be harmful and one form is accredited with producing the special flavor of some English beers.

The forms included under *Mycoderma* resemble yeast in shape but produce little or no alcohol, are strongly aerobic and do not produce endospores. Their most noticeable characteristic is that they grow only on the surface of the liquid, where they produce a thick film. They cause complete combustion of the alcohol and other organic matters, making beer and wine vapid and finally spoiling them.

Culture of Yeasts

Pure Cultures.—Yeast can be properly studied only in pure cultures. The media used are either the liquids in which the yeasts are to be used such as wort, cider, grape juice, or a special medium devised for a special investigation. An example of the latter is Laurent's medium:

Ammonium sulphate,	4.71 g.
Potassium phosphate,	0.75 g.
Magnesium sulphate,	0.10 g.
Water,	1 L.

To this is to be added any carbohydrate to be studied. Media may be made solid by the addition of gelatin or agar.

Pure cultures can be made, rarely, by inoculation from a naturally pure source, such as the sporangium of a *Mucor*.

Physiological Separation.—The first attempts at purifying mixed cultures were by means of physiological differences. Pasteur freed yeast from bacteria by growing it in a medium containing 2 per cent. of tartaric acid. Effront used fluorides in the same way. These methods may be made more effective by repeated transfers of the culture. Each transfer will contain a larger proportion of the form most suited to the conditions, until finally a pure culture may be obtained. The principle of these methods is of great use in practical fermentation, but is of little use in rigidly separating forms. Methods of general application for the latter purpose must be such that a single cell can be isolated in a sterile medium and a culture propagated from this single cell.

Separation by Dilution in Liquid Media.—A mixed culture is diluted with sterilized water until on the average every two drops contain one cell. A large number of flasks of a sterilized nutrient medium is then inoculated from the dilution, one drop in each flask. If the dilution has been properly made, about half of the flasks will remain sterile and half will show growth. Many or most of the latter will contain pure cultures.

Separation by Dilution in Solid Media.—If we dip a sterilized platinum wire into a mixed culture and then draw it repeatedly over the surface of a solid culture medium

such as a slice of sterilized potato or a layer of nutrient gelatin in a petri dish we will get a series of *streak cultures*. The first of these will develop a strong growth of mixed forms. The last will show more and more isolated colonies until some of them will show only a few, some of which may be pure cultures.

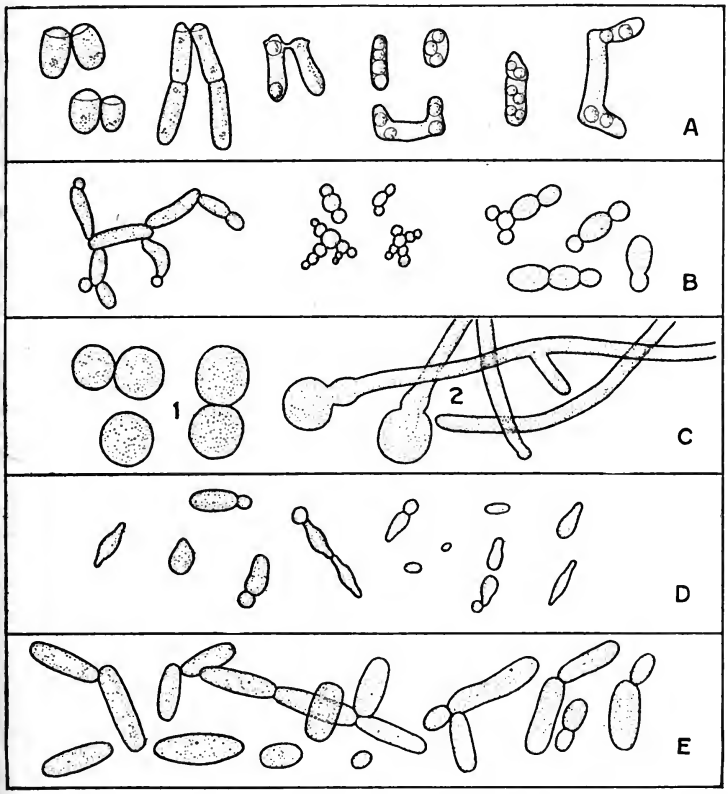


FIG. 55.—Wild and pseudo yeasts. *A*, *S. pombe*. (*After Lindner*). *B. Torulæ*. (*After Pasteur.*) *C*, *Mucor*, (1) spores; (2) germinating spores and mycelium. *D*, *S. apiculatus*. *E*, *Mycoderma vini*. (*After Biolctti.*)

The most useful method of separation and one which is applicable to most cases is that of *plate cultures*, first used by Koch and improved by others. In this method a drop of the mixed culture is thoroughly distributed in 10 to 20 c.c. of liquefied nutrient gelatin or agar. A drop of this mixture is then diluted in the same way in another portion of the same medium. This process is continued until the requisite

degree of dilution is obtained. The various portions of nutrient gelatin are then poured, with precautions against outside infection, on glass plates or more conveniently into petri dishes. On cooling and solidifying, the gelatin imprisons every cell, each of which on growing gives rise to a colony. It has been found that in practice a small percentage of these colonies may arise from two adhering cells and thus fail to be pure culture.

Hansen's modification of the method is intended to obviate this uncertainty. By making the dilutions in the way described for liquid media, a drop of gelatin containing only one cell is obtained, placed on a cover-glass over a culture slide and, by direct observation, the presence of a single cell verified. The development and multiplication of this cell can be watched.

Differentiation of Yeasts.—With magnifications of 300 to 500, yeast cells can be examined conveniently. Contamination with bacteria and molds of special form can be detected, but otherwise a simple microscopic examination is of little value in determining the purity of a culture. Some information regarding the health, nutrition and vitality of the yeast may be obtained and the form of the spores is of some value in distinguishing species. Yeast cells vary in size as much as in form but under standard conditions each variety will show a certain normal range of dimensions.

If a young, vigorous yeast, in a favorable liquid culture medium, is allowed to remain at rest at a suitable temperature with full access to air and protection from contamination, a growth of cells on the surface will usually take place. This growth may extend over the whole surface (*film formation*) or may be restricted to the edges (*ring formation*). This growth occurs at once with a few species (*S. membranæfaciens*) or at the end of several days (*S. ellipsoideus II*) or may require several weeks. The time and optimum temperature of film formation have been used as descriptive characters.

All the morphological and cultural characteristics of yeast are insufficient for diagnostic purposes and must be supplemented by the physiological characteristics such as their action on various sugars and other carbohydrates.

CHAPTER IV

BACTERIA*

The bacteria naturally fall into quite distinct groups or orders—the true bacteria and the sulphur bacteria.

A portion of the true or *Eubacteria* together with the sulphur form, are designated as the higher bacteria. The forms usually spoken of as bacteria belong to the group of lower bacteria, and when the word "bacteria" alone is used reference is usually made to the lower bacteria. These constitute a group of microörganisms quite distinct and characteristic, while the higher bacteria form links, as it were, between the lower bacteria and other closely related microörganisms. The morphology of the two groups will need to be discussed separately.*

Forms of Lower Bacteria*

Fundamental Form Types.—The forms of bacteria are exceedingly simple. They are either spheres, straight rods, or bent rods (spiral). In the spherical form they are known as *cocci*, or *micrococci* (sing. *coccus* or *micrococcus*). The straight rods are *bacilli* (sing. *bacillus*) and the bent rods are *spirilla* (sing. *spirillum*).

Fig. 56.—Types of micrococci. (*After Williams.*)

Fig. 57.—Types of bacilli. (*After Williams.*)

*Prepared by W. D. Frost, with cytology by A. Guilliermond.

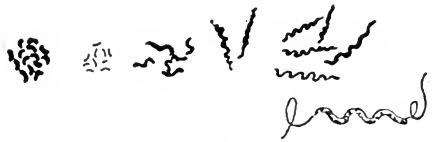


FIG. 58.—Types of spirilla. (*After Williams.*)

GRADATIONS.—The difference between these fundamental form types is frequently very slight. It becomes a very difficult matter, for instance, to distinguish at times between the micrococcus and the bacillus. There is a number of bacteria, and among them the well-known example of *B. prodigiosus*, that are described at one time by one investigator as micrococci and at another time, or, by another investigator, as bacilli. The pneumonia germ is also another illustration of an organism that occupies a dual position. Migula has suggested a method of differentiating these which will be discussed under a later head. The bacilli pass almost imperceptibly into the spirilla. The cholera bacillus of Koch is in reality a spirillum.

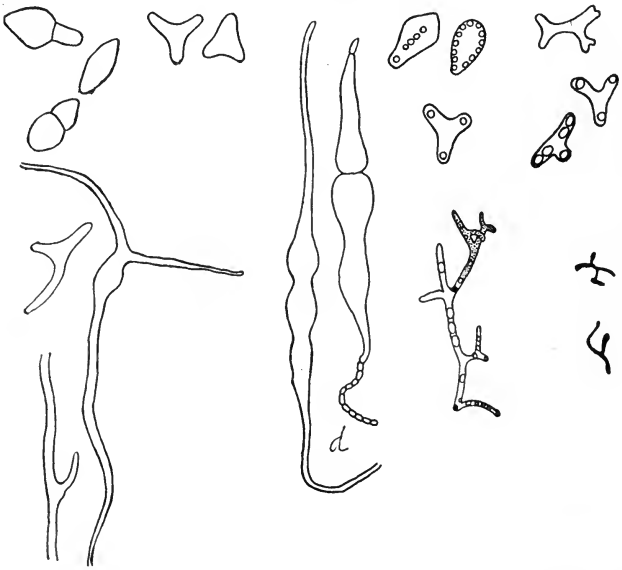


FIG. 59.—*Involution forms.* Here are illustrated unusual forms of *B. subtilis*, water bacteria, *Bact. aceti*, *Bact. pasteurianum*, bacteroids in root nodules, *Bact. tuberculosis*, *Bact. diphtheriæ*. (*After Fischer from Frost and McCampbell.*)

INVOLUTION FORMS.*—The forms of bacteria are quite constant under normal conditions, but very frequently they show abnormal or bizarre shapes. These are known as involution forms (Fig. 59). It is sometimes suggested that these involution forms represent another stage in the developmental history of the organism, and upon this supposition certain bacteria which very regularly show these involution forms have been classified as belonging to a different suborder from that in which the lower bacteria are placed. The ordinary view of the involution forms is, however, that they are degeneration forms, that they correspond, in other words, to the halt and maimed in society and are to be accounted for by the fact that they are deformed by their own by-products. In fact, it is quite probable that they are autogenic. Involution forms are very likely to occur in artificial culture and are much more common with some species than with others. (See page 98.)

SIZE*

The bacteria were formerly spoken of as the smallest of living things, but since the recognition of the ultramicroscopic organisms it is necessary to be somewhat more specific in characterizing their dimensions. The unit of measurement in microscopy is the micron (μ), or micromillimeter. This is .001 mm. or approximately 1/25000 of an inch. Applying this unit to the bacteria we find that the micrococci and the short diameter of the bacilli and spirilla average about 1μ. The micrococci vary in diameter from a small fraction of a micron to three or four microns in diameter. The bacilli are sometimes very small, as the influenza bacterium with a width of 0.2μ and a length of 0.5μ, and sometimes very large as, for example, the *Bact. anthracis* with a width of 1.2μ and a length of 5.20μ. The spirilla average about 1.0μ in diameter but may be as long as 30μ–40μ.

MOTILITY*

When bacteria are viewed under the microscope in a living condition many of them are seen to move. This movement may be one of two kinds. In some cases it is progressive, the individuals move about from one part of the field of the microscope to another and change their rela-

*Prepared by W. D. Frost.

tive positions. In other cases the movement is vibratory, the bacteria move back and forth but do not progress or change their relative positions to any extent. This latter form of movement is known as *brownian movement*, because it was first described by Brown.

Brownian Movement.—This movement is probably caused by the impact of the molecules of the suspending medium and for this reason is sometimes called molecular movement. It is not characteristic of bacteria, or indeed of life, but is shared by many small microscopical objects when suspended in a fluid medium. Most beautiful examples of brownian movement can be seen by suspending granules of India ink or carmine and examining them under the microscope. This brownian movement is to be sharply differentiated from *vital movement* which is possessed by some bacteria.

Vital Movement.—As already indicated, bacteria have the power of independent movement due to inherent vital power. Only a few of the micrococci are motile, while many of the bacilli and spirilla are. This movement is a change of position and is caused by certain protoplasmic processes which these bacteria possess, known as *cilia* (sing. *cilium*) or *flagella* (sing. *flagellum*). The fact of motility or non-motility of an organism is of considerable value to the systematist. It is determined by examination in a *hanging drop*. At times, however, it varies so little from the brownian movement that it is difficult to tell whether a particular organism or culture does or does not possess vital movement. An opinion can be more definitely formed at times if some chemical producing an anæsthetizing effect on the bacteria is introduced into the examining medium. In case the organism is actually motile its movement will be altered by the anæsthetic but in case it is merely a brownian movement there will be no change.

Organs of Locomotion.—The protoplasmic threads referred to as the organs of locomotion are known as flagella, or cilia. The difference between the cilium and flagellum is the fact that a cilium has a simple curve while a flagellum has a compound curve, like a whip lash. Most of the bacteria possess flagella rather than cilia. The size, arrangement, etc., of these flagella are constant and characteristic of a particular organism. Their structure and arrangement, therefore, will be discussed later.

Character of Movement.—Different bacteria exhibit different kinds of movement. Some dart forward with great rapidity, others

move slowly; some move in straight lines, others wobble, but any particular character is quite constant and many of the bacteria may be recognized by their peculiar movements.

RATE.—The rate at which the bacteria travel when they possess vital movement varies greatly. Some of them move very fast, others very slowly. Many of them appear to move with wonderful rapidity. Van Leeuwenhoek, when he first saw these moving bacteria, said that they traveled with such great rapidity that they tore through one another, but it must be borne in mind that under the high powers of the microscope the rate of movement is magnified to the same extent as the object, and that in reality the rate of movement is not excessive. When compared to their size, the rate of movement is probably little greater than that of a trotting horse and considerably less than that of a speeding automobile or a railroad train.

REPRODUCTION*

Reproduction among the bacteria is largely asexual and takes place ordinarily by what is known as binary fission. In addition to this a

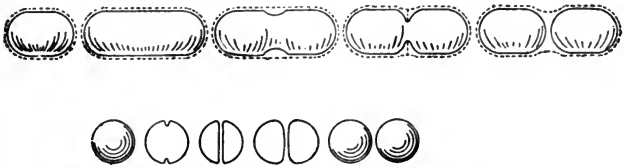


FIG. 60.—The division of bacterial cells (diagrammatic). (*After Novy.*)

number of bacteria go into a resting stage, or produce spores. The spore formation is not, however, a method of multiplication, because usually only a single spore is formed in a cell, but serves to tide the organism through unfavorable conditions.

VEGETATIVE MULTIPLICATION.—This is accomplished by means of binary fission (Fig. 60). When a bacterium has reached maturity, fission begins. Division begins by an invagination of the protoplasm in the middle of the cell, which proceeds until the cell protoplasm is completely separated. The cell wall then grows in and finally splits forming the two ends of the new cells. These new cell walls are formed

*Prepared by W. D. Frost.

at right angles with the long axis of the cell in the case of the bacilli and spirilla, except in rare instances. In the case of micrococci, the throwing of the cell wall across one diameter is quite as economical as any other and may therefore proceed in any direction. Migula makes a considerable point of the fact that bacilli and spirilla elongate before division and micrococci divide before they elongate; this would be the criterion which he would use to separate these two-form types. A generation among the bacteria is from one division of the cell to another. This is sometimes very short, in fact, only twenty to thirty minutes. Many of the bacteria after half-an-hour's time have grown from newly formed cells to maturity and are ready to divide again. This makes it possible for bacteria to multiply with very great rapidity, and if we know the length of the generation in a particular bacterium it would be easy enough to estimate the rate of multiplication, at least theoretically. It would be only a matter of geometrical progression. It is of course quite impossible for the bacteria to maintain their theoretical rate of growth for any length of time, but, practically, they grow with enormous rapidity, as is shown in cultures and by the changes which they bring about in nature, such as the production of fermentation and the generation of toxin.

Spore Formation.—A considerable number of bacteria form spores within the cell. Because they are formed within the cell they are spoken of as *endospores*. Endospores are formed by the bacilli and the spirilla, but not by the micrococci. Their chief value to the cell is their ability to resist unusual conditions, and to enable the individuals of a species to pass through unfavorable conditions which to the ordinary vegetative form of the cell would prove disastrous. At the maturity of the cell, spore formation may begin. It is an open question whether spore formation occurs as a regular stage in the life history of an organism, or is produced only under the stimulus of unfavorable environmental conditions. Both theories have their advocates. The first evidence of spore formation in the cell is a granulation of the protoplasm of the cell. As spore formation proceeds the granules become larger and collect at one portion of the cell. These granules then fuse to form the spore, which soon surrounds itself with a spore wall. At times the spore is smaller than the mother cell and is formed without changing the shape of the cell. At other times it is larger than the mother cell and causes a bulging of the latter. The position

of the spore in the cell varies (Fig. 62). In some species it is *equatorial*, in others it is *polar*, and in still others it has an *intermediate* position between equatorial and polar. When the spore is larger than the mother cell and is situated equatorially it causes the cell to bulge with the formation of a barrel-shaped organism, a *clostridium*. If the spore is situated at the poles and is larger than the mother cell, a *capitate* or *drum-stick* bacillus is produced. When the spore is smaller than the mother cell and the cells form in chains, there is frequently a tendency for the spores to be formed in opposite ends of contiguous cells of the chain so that they appear in pairs. The reason for this is not understood.

The endospores possess remarkable powers of resistance due to the concentrated character of the protoplasm, or to the character of the

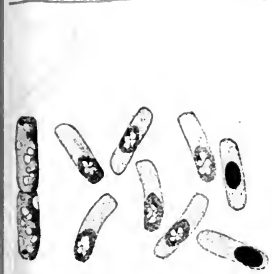


FIG. 61.

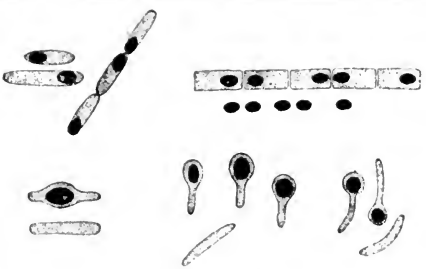


FIG. 62.

FIG. 61.—The formation of spores. (*After Fischer from Frost and McCampbell.*)
FIG. 62.—Spores and their location in bacterial cells. (*After Frost and McCampbell.*)

spore wall. The resistance here may be due to the structure of the wall itself or to the chemical substances which it contains. It is readily conceivable that the presence of certain fatty acids, or higher alcohols, might give the spore its remarkable resistance. These spores are very resistant to desiccation; they have been preserved in a dried condition for many years. They are also very resistant to the action of heat; some forms are known to withstand a temperature of boiling water for as long a time even as sixteen hours. They are resistant also to chemicals and the action of sunlight. Although in some cases, as pointed out by Marshall Ward, the very chemical substances which furnish them the powers of resistance toward environmental factors may be broken up under the influence of sunlight, forming poisons so that the spore is killed more readily than the cell would be.

When these spores are brought under favorable conditions of moisture, temperature, and food supply, they germinate. There are several types of germination (Fig. 63). In some cases the spore wall ruptures at the pole and the young cell emerges so that its long axis is in the same direction as the long axis of the spore. In another type the spore ruptures equatorially and the young cell emerges with its long axis at right angles to the long axis of the spore. In still another type the spore swells and the young cell absorbs the wall of the spore.

In the lower bacteria only a single spore is formed in a cell. In the case of the higher bacteria, however, a number of spores may be formed at the distal end of the filament. These are spoken of as *gonidia*, and possess properties similar to those of the endospores.

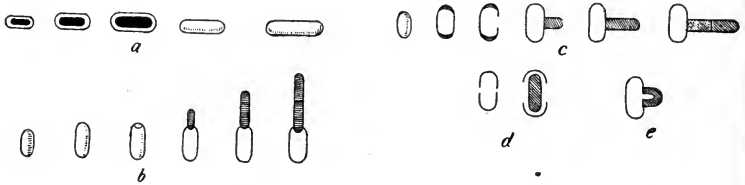


FIG. 63.—*Spore germination.* *a*, direct conversion of a spore into a bacillus without the shedding of a spore-wall (*B. leptosporus*); *b*, polar germination of *Bact. anthracis; c*, equatorial germination of *B. subtilis; d*, same of *B. megaterium; e*, same with "horse-shoe" presentation. (*After Novy.*)

In some cultures of bacteria, as for example in the micrococci, certain cells seem to be larger and different from the other cells. In a streptococcus filament, certain cells suggest to the observer the *joint spores* of the algæ and have therefore been spoken of as *arthrospores* or *joint spores*. There is, however, no evidence of an experimental nature, which warrants the belief that these cells are in reality spores, and it must be said that at the present time the presence of arthrospores among the bacteria is purely hypothetical.

CELL GROUPING*

Bacteria rarely occur singly but usually in groups. These cell aggregates are frequently very constant and quite characteristic of the

*Prepared by W. D. Frost.

organism possessing them. They are of sufficient definiteness and constancy to be used by the systematists in characterizing large groups.

Cell Aggregates among the Micrococci.—The grouping of micrococci depends upon the plane of division and also upon the cohesion of the cells. Since it is quite as economical for the micrococcus to divide in one direction as another, it is possible for a number of different cell groupings to occur. Whatever the direction of the dividing walls, it is usually quite constant; if a particular species of micrococci has its planes of division parallel, there will be formed chains of micrococci. In some cases the cohesion is slight and only two cells remain attached to each other, forming what are ordinarily known as *diplococci*. There is a considerable number of very well-known bacteria that are diplococci (Fig. 64). If the cohesion is stronger, we have chains of micrococci or rosaries formed which are known as *streptococci*. Well-known and very important bacteria are grouped in this way. In other micrococci the cell wall is not formed continuously in parallel planes but in

Fig. 64.—Division forms of micrococci. *a*, *Diplococcus*, perfect form with flattened opposed surface (*gonococcus*), lanceolate form (*pneumococcus*); *b*, *streptococcus*; *c*, consecutive fission yielding a *tetrad*; *d*, *sarcina* form resulting from division of tetrad *c*; *e*, *staphylococcus*. (*After Novy.*)

planes which alternate at right angles to each other. In this way cell aggregates occupying two dimensions of space are formed. These are known as *tetracocci*, or *merismopedia*. Still again, the planes of division may proceed at right angles to each other in three dimensions of space. In this case packets are formed which are known as *packet cocci*, or *sarcinæ*. Another group of the micrococci occurs, known as the *staphylococci*, so called because they are arranged in irregular bunches, like a bunch of grapes. This arrangement may be due to the fact that these micrococci divide in many different planes, or because during the course of their growth their arrangement is changed.

Cell Aggregates among the Bacilli.—In the case of the bacilli, one diameter is usually considerably shorter than the other, so that nature almost invariably throws the new cell wall across the bacilli

at right angles to their long axis (Fig. 65). There is, therefore, only one arrangement or cell grouping possible, and that is end to end, so that *streptobacilli* are formed. When arranged in pairs, the designation is *diplobacilli*. The length of the chains appears to depend not

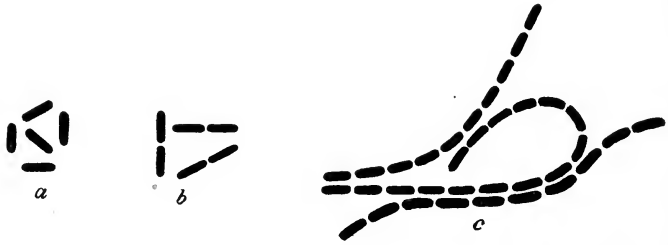


FIG. 65.—Division forms of bacilli. *a*, Single; *b*, pairs; *c*, in threads. (*After Novy.*)

only upon the cohesion of the bacilli but also upon the shape of the end; those which have square ends frequently have very long chains, while those with rounded ends have short chains or occur singly.

CELL AGGREGATES AMONG THE SPIRILLA.—The same kind of arrangement is maintained among the spirilla.

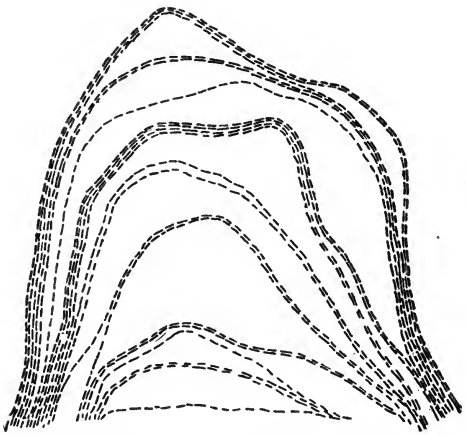


FIG. 66.—Threads of *Bact. anthracis.* (*After Migula.*)

ZOOGLŒA.—Some of the bacteria secrete a mucilaginous substance which causes the cohesion of the cells frequently in considerable number. This aggregate of cells may assume some characteristic appearance and

a great many attempts have been made by systematists to make use of this in indifferentiating species. These zoogloœic masses usually assume the forms of pellicles, but their value as diagnostic features is not great. The formation of zoogloœa is very frequently only a stage in the life history of an organism.

The Cytology of Bacteria

* The typical cell, such as that of a higher plant or animal, is made up of cytoplasm surrounded by a cell wall. The cytoplasm contains a nucleus. There are also frequently present other evidences of structure in the cytoplasm, such as nucleolus, polar bodies, etc. In addition to these there may be appendages, such as the cilia or flagella. In the case of bacterial cells, we find most of these structures present, such as cell wall, cytoplasm, and appendages.

General Consideration of Cytoplasm and Nucleus.*—The cytoplasm of the bacterial cell is similar to the cytoplasm of other cells except that chemical analyses seem to show that it contains a higher

Fig. 67.—Plasmolytic changes. (*After A. Fischer.*) *a*, Cholera vibrio; *b*, typhoid bacillus; *c*, *Spirillum undula*. (*From Novy.*)

percentage of nitrogen. As viewed under the microscope, in either an unstained or stained condition, it appears as a homogeneous mass filling the entire cell and rarely showing any evidence of structure. Ordinary stains, such as are used in animal and plant histology, fail to reveal the presence of a nucleus, the whole cell being usually uniformly stained with those stains generally characterized as nuclear stains. When these stains are applied to some bacteria, particularly at certain stages of their growth, certain parts stain more readily than others, and we get either what is known as a bi-polar stain or polar granules. In the first case, the ends of bacilli are stained more deeply than the center so that the cells appear very much as diplococci. This

* Prepared by W. D. Frost.

bi-polar stain is characteristic of such organisms as the bacterium of chicken cholera or the bacterium of bubonic plague. The polar granules are frequently seen in the diphtheria bacterium and may be located at the poles and also at the center. In this germ and in some others it is possible, by special staining, to give the granules a different color from the rest of the organism. In this case these bodies are spoken of as *metachromatic granules* which are considered later under "Reserve Products." The presence of these granules might possibly be explained upon the theory that the cells are plasmolyzed (Fig. 67). As a result of plasmolysis the protoplasm of the cell is drawn away from the cell wall and concentrated in areas which would very well explain the appearances. And it seems likely also that the methods employed in staining might lead to plasmolysis, but the metachromatic granules can hardly be explained upon this supposition.

The cytoplasm of the bacterial cell is slightly refractive. It is colorless except in a few cases in which the green coloring matter, like chlorophyl, is present, as, for instance, *Bact. viride* and *Bact. chlorinum*. In the purple sulphur bacteria, the coloring matter *bacteriopurpurin* is present. The bacterial cytoplasm contains vacuoles at times.

Minute Considerations of Cytoplasm and Nucleus.*—The question of the cytology of bacteria has long excited the curiosity of biologists. It is indeed of great importance from many points of view. In the first place, we are interested to know whether bacteria are ordinary cells having a nucleus; or whether, as some maintain, they lack entirely a nuclear element and are an exception to the rule elsewhere established. Moreover, the cytologic study of bacteria may furnish useful knowledge concerning the phylogeny and taxonomy of these organisms, a matter not yet solved. Finally, we may hope that it will throw light upon some problems of a physiological or pathological nature.

Unfortunately this study is very delicate, because of the extreme minuteness of the bacterial cells, so that in spite of the large number of researches which it has incited in the last twenty-five years, it is to this day a matter of controversy.

At present three theories are held by authors relative to the interpretation of the general structure of bacteria. We will examine these

*Prepared by A. Guilliermond.

three theories one by one, endeavoring to determine which one, in our opinion, seems most probable.

One of these theories claims that bacteria are cells of very primitive organization lacking nucleus and consisting simply of cytoplasm with vacuoles. The cytoplasm contains many stainable granulations, but these represent products of nutrition. Such an opinion scarcely accords with our knowledge of the constitution of the other *Protista*, in all of which the existence of a typical nucleus, or at least of chromatic elements replacing the nucleus, has been established. This view has not, therefore, had many supporters.

Another theory maintains that bacteria have a typical nucleus and are in no way structurally different from ordinary cells. This opinion was suggested by Arthur Meyer, who claims to have succeeded in differentiating, in a great many bacteria, granules which fix nuclear stains, and of which one or often several appear in a cell. These granules he would consider nuclei. It seems to be established, however, that the majority of the elements noted by Meyer are not nuclei, but reserve products common among the *Protista* and known as metachromatic corpuscles.

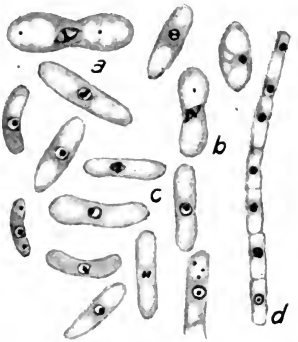


FIG. 68.—*Bacterium gammari* and a filamentous bacterium from the intestine of *Bryodrilus*. (*After Véjdowsky.*)

Véjdowsky's efforts have resulted in much weightier proofs in favor of the existence of a true nucleus. In the *Bacterium gammari*, a species discovered by him in the sections of a little fresh water crustacean, *Gammarus zschokkei*, Véjdowsky has been able to demonstrate in each cell a typical nucleus which is always present. This nucleus appears very clearly; it consists of a colorless nucleoplasm surrounded by a membrane and by one or two karyosomes (Fig. 68). The author had the good fortune to ascertain in several cases karyokinetic representations of the division of this nucleus (*a*, *b*, *c*). In short, the presence of this nucleus is indisputable.

The same author discovered a similar structure in a filamentous bacterium found in the digestive tract of an *Annelida* (*Bryodrilus ehlersi*) (Fig. 68, *d*).

These conclusions are positive, but the species observed by Vejdowsky are not well-defined bacteria, and may be thought to belong to the molds rather than to the bacteria. It has also been said, not without reason, that *Bact. gammari* might be a yeast of the genus *Schizosacchromyces* and that the filamentous bacterium studied by Véjdowski seems to resemble a filamentous mold.

However this may be, one of Véjdowsky's pupils, Mencl, has endeavored to apply these conclusions to other bacteria, which are well-defined, notably *B. megatherium*, but has only succeeded in bringing forth proofs which are much less convincing of the existence of a nucleus. The author strived to discover a nucleus, but this organ is not constant and does not show the structure of a true nucleus.

Both Kruis and Rayman have discovered a nucleus in different bacteria (*B. mycoïdes*, *radicosus*, etc.). This nucleus appears only in very young cells; it is not found in older cells, and seems (like the nucleus noted by Mencl) to represent merely the incipient transverse septum which fixes stains well at the beginning of its formation and in some ways resembles a nucleus.

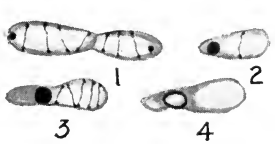


FIG. 69.—*Bacillus megatherium.* (*After Penau.*)

The studies of Penau, who also endeavored to prove the existence of a typical nucleus in bacteria, were no more successful. In *B. megatherium*, he describes the following phases. In the youngest cells he observes a stage where the cytoplasm is very dense and uniformly stained, without a trace of differentiation. Immediately succeeding is a phase where the cytoplasm becomes less chromatic and is filled with vacuoles. At this point the author finds in each cell a tiny granule (Fig. 69, 1), homogeneous and easily stained, situated at one of the poles of the cell, very near the membrane. This granule he considers to be a nucleus. Moreover, in the cytoplasmic web he observes a series of stainable granules connected by slender trabeculæ, thus forming a kind of network which he likens to mitochondrial and chromidial formations. At the time of sporulation, Penau finds an increase in the size of the nucleus (Fig. 69, 2 and 3) which changes to a large granule; this is soon surrounded by a membrane and becomes the spore (4), which is therefore formed mostly of chromatin.

The same author discovers a very different structure in *Bact. anthracis*. Here, after a stage of undifferentiated structure which

characterizes the youngest cells, follows a phase where the cytoplasm becomes alveolar. At this time, at one of the poles of each cell, appears a very large homogeneous granule which Penau regards as a nucleus. This nucleus, however, has only an ephemeral existence and quickly undergoes a cytolysis during which it disintegrates. The disintegration products then impregnate the trabeculæ of the cytoplasm and the nucleus becomes diffuse. In a last phase which corresponds to sporogenesis, the chromatin which impregnates the cytoplasm is partly condensed at one of the poles, where it forms first a mass of grains, then a large granule which changes to a spore.

Nothing is less conclusive than these results, since the author cannot discover an homologous structure in the different species which he studies, and since the nucleus which he describes is only a transitory organ not showing the distinguishing characteristics of a nucleus.

To prove the existence of a nucleus in bacteria, it is necessary to show a nucleus with a differentiated structure, the constant presence of the nucleus, and to follow the division of this organ during the cellular separation. So far no one has apparently been able to differentiate such an organ in well-defined bacteria. We must conclude, therefore, that with the exception of the results obtained by Véjdowsky, all observations so far gathered in favor of the existence of a typical nucleus in bacteria are by no means convincing.

The third theory asserts the existence of a *diffuse nucleus* in bacteria. It was first suggested by Weigert and more carefully formulated by Bütschli. This author describes in a certain number of *Sulfo-bacteria* of large size, *Beggiatoa*, *Chromatium*, a kind of *central body* occupying

FIG. 70.—1. *Chromatium okenii*. 2. *Beggiatoa alba*. These two bacteria have a central body containing chromatic grains and considered by Bütschli as the equivalent of a nucleus. (*After Bütschli.*)

nearly the whole volume of the cell and consisting of an alveolar cytoplasm of highly stainable web, containing within its knots numerous chromatic granulations (Fig. 70). The remainder of the cell consists

of a thin cytoplasmic layer, less easily stainable, surrounding the central body. Bütschli compares this structure with the one which has been demonstrated in the *Cyanophyceæ*, and claims that the central body represents the equivalent of a nucleus. It would be a sort of large nucleus occupying most of the cell, not bounded by a membrane, and scarcely distinct from the cytoplasm. This structure has recently been verified in *Chromatium okenii* by Dangeard. The *Sulpho-bacteria*, however, are organisms morphologically entirely distinct from ordinary bacteria, and are apparently directly related to the *Cyanophyceæ*. Such a structure is not found in other bacteria, in which it is impossible to demonstrate a central body and in which, one must admit, the nucleus is still more diffuse.

To Schaudinn we are indebted for the most exact observations in favor of the theory of the diffuse nucleus. He had the good fortune to discover in the intestine of the cockroach, *Periplaneta orientalis*, a bacillus of very large size which he named *B. bütschlii*. It is the largest bacillus known at present (4μ wide), and lends itself readily, therefore, to cytological studies. His minute observations have shown that there is no nucleus. The cells enclosing a finely alveolar cytoplasm, whose net contains many small grains which take nuclear stains (Fig. 71, 1–6).

At the time of sporulation the chromatic grains increase in size (Fig. 71, 7–9), then gather at the center of the cell in a kind of axial wreath (Fig. 71, 10). The two extremities of this wreath quickly swell with an accumulation of chromatic grains and form two granular masses, one at either pole. These two masses form the beginning of the two spores, for each cell forms two spores (Fig. 71, 11 and 12). The grains which compose these two rudiments then condense to form two large homogeneous granules (Fig. 71, 13) which strongly resemble nuclei and which Schaudinn considers to be such. Around these two granules is soon condensed a thin cytoplasmic zone which in turn is separated from the surrounding cytoplasm by a membrane (Fig. 71, 13). Henceforth the spores cannot be stained by ordinary means because of the thickness of their membrane which prevents the penetration of stains (Fig. 71, 14). The granules of the wreath, which joined the two rudiments of spores, gradually disappeared as well as the cytoplasm, while the spores increased in size. Then the sporangium ended by breaking and setting free the two spores. Germination con-

sists simply of a swelling of the spore, then the formation of a small rod which issues from the spore and forms a septum for itself (Fig. 71, 15 and 16). As soon as the spore germinates, the nucleus ceases to exist as a morphologic entity; it is scattered in the cytoplasm in the form of little grains.

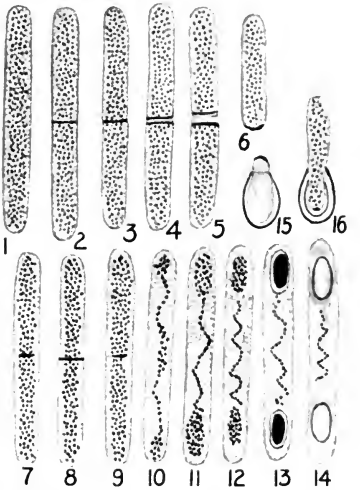


FIG. 71.—*Bacillus bütschlii.* 1–16, Vegetative cells and their division. 7–9, Beginning of sporulation: the cells about to sporulate are partitioned off crosswise; then the septum thus formed is absorbed, at which time sporulation begins. Schaudinn considers this partitioning off followed by fusion of the two daughter cells as a rudimentary sexuality. 10–13, Formation of the beginnings of the two spores, at the poles of the cell. 14, Ripe spores. 15–16, germination of the spore. (*After Schaudinn.*)

In another bacillus smaller in size (*B. sporonema*), Schaudinn has found an analogous structure only at the time of sporulation; he does not prove the formation of an axial filament but only the condensation of a portion of the chromatic grains into a large granule which forms the beginning of the spore (Fig. 72).

By the fact that in these two bacilli the beginning of the spores appears as a granule equivalent in some respects to a nucleus and resulting from the condensation of a portion of the stainable grains, Schaudinn is led to believe that these grains are composed of chromatin and represent a kind of diffuse nucleus.

These results have been confirmed by our studies of a large number of endospore bacilli (*B. megatherium, radicosus, mycoides, asterosporus, alvei*). Upon examination at the very outset of their development, these bacteria present a homogeneous appearance and are uniformly

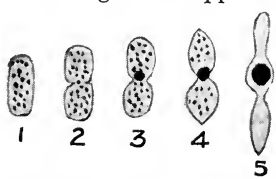


FIG. 72.—*Bacillus sporonema.* 1. Cell about to sporulate. 2, This cell grows narrow at the center, as if it were going to be divided (Schaudinn regards this pinching together which afterward disappears (5), as the vestige of an ancestral sexuality like that of *B. bütschlii*). 3–5, Formation of the beginning of the spore. (*After Schaudinn.*)

stained with no great differentiation, explicable by the density of the cytoplasm or by a special condition of the membrane. At this stage the cells are in the process of active divisions, after which the transverse septa are formed as follows: On the side walls of the bacillus appear two small granules which take some stains (Fig. 73, 1). These soon

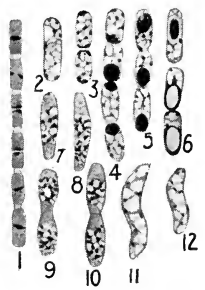


FIG. 73.—1–10, *Bacillus radicosus.* 1, Beginning of development. 2–3, Cells at the end of eight hours; 4–6, sporulation. 9–10, Cells in which the chromatic grains are located in the middle in a mass slightly resembling a nucleus. 11–12, *Spirillum volutans.*

disintegrate at the center of the cell to form a thin band marking out the two daughter cells and forming the beginning of the transverse septum. This strongly resembles a nucleus and has apparently been considered as such by a number of authors (Rayman and Krius, Mencl).

Toward the eighth hour of development, the cells show clearly their

structure which is changed in appearance; the cytoplasm vacuolizes and ends by displaying a fine alveolar structure. The web contains in its knots small, highly stainable granules (Fig. 73, 2 and 3). In some cases (cultures on special media for example), there is noticeable a localization of these granules at the center of each cell, forming a granular region which recalls somewhat the appearance of a large nucleus and which is separated into two portions at the time of the cellular division as if it were indeed a true nucleus (Fig. 73, 7 and 10).

These granules fix the nuclear stains, and it seems permissible to consider them chromatic in nature.

At the time of sporulation there forms at one of the poles of the cell a small oval mass, easily stained, which is like a nucleus in appearance (Fig. 73, 4 and 5). This results from the condensation of part of the chromatic granules of the cytoplasm, gradually grows larger, and changes to a spore. When the spore has reached a certain size, it is surrounded by a membrane which prevents the penetration of ordinary stains (Fig. 73, 6); it appears then like a large colorless sphere in the stained cytoplasm of the cell (Fig. 73, 6).

At no stage of the development have we observed the least trace of a nucleus. May there be a nucleus which our present technic would not enable us to differentiate? That has seemed to us scarcely probable, for if this nucleus existed, it would certainly be visible in a species as large as *B. bütschlii* and would not have escaped Schaudinn. The most reasonable hypothesis, the one which we have adopted, is to consider like Schaudinn that bacteria contain chromatin more or less mingled with cytoplasm, differentiated in the case of small grains and condensing at the time of sporulation to form the spore which would *consist principally of chromatin.* The cells of bacteria would accordingly have a very primitive structure.

Granted the clearly demonstrated existence of this particular structure in the *Cyanophyceæ*, there is no reason for not admitting that the nucleus, very rudimentary in the *Cyanophyceæ*, might be even more so in bacteria, being reduced to a diffuse nucleus consisting of chromatic grains scattered in the cytoplasm.

These observations have, moreover, received a series of new confirmations by the labors of a great many authors (Swellengrebel, Ruzicka, Ambrez, etc.) and especially by the later researches of Dobell.

The latter investigator discovered, in the intestines of frogs and toads, a large bacillus (2μ wide) almost as large as *B. bütschlii*, and named it, *B. flexilis*. This species shows exactly the same cytological characteristics as *B. bütschlii* (Fig. 74).

Through a study of a number of different bacteria found in the intestine of toads, frogs and lizards, Dobell has endeavored to show that this diffuse nucleus is not original, but derived from the retrogression of a more highly differentiated nucleus.

Thus in various micrococci he was able to show in each cell the existence of a central stainable granule, dividing by constriction at the time of cellular division, and which he regards as a nucleus (Fig. 75,

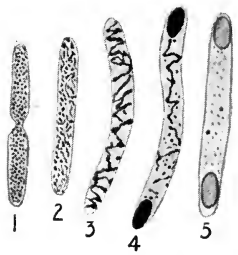


FIG. 74.

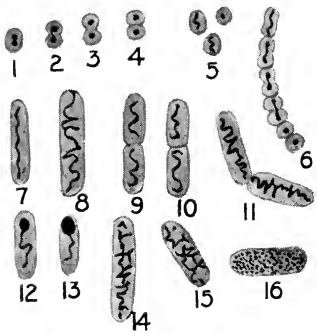


FIG. 75.

FIG. 74.—*Bacillus flexilis*. 1, Beginning of the division of a cell about to sporulate (vestige of sexuality). 2, Disappearance of the incipient division. 3, Formation of the chromatic axial filament. 4, Formation of the beginning of two spores. 5, Ripe spores. (*After Dobell.*)

FIG. 75.—Various bacteria, showing the successive types of the retrogression of the original nucleus and its transformation to a diffuse nucleus. (*After Dobell.*)

1–5). In other cocco-bacillary species of bacteria characterized by spherical shape capable of elongation, Dobell discovers a similar nucleus in the spherical cells. When the cell lengthens and assumes the appearance of a bacillus, this nucleus changes to a spiral axial filament (Fig. 75, 5 and 6).

In various bacilli the same author demonstrates a filament which is ever present (Fig. 75, 7–11). The spore results from the condensation, at one of the poles, in the shape of a large chromatic granule, of part

of the grains which compose this filament (Fig. 75, 12 and 13). An interesting variation of this structure is found in *B. saccobrinchi.* In this bacillus is noticed first an initial stage where the nucleus is represented by an axial filament quite similar to that of *B. spirogyra* (Fig. 75, 14). In the course of development, however, this filament resolves itself into a great many grains which scatter through the cell (Fig. 75, 15 and 16). The nucleus then becomes diffuse. Part of this diffuse nucleus next condenses at the time of sporulation into a large chromatic grain which forms the beginning of the spore. Finally, in other bacilli, Dobell finds in the whole development no more than a diffuse nucleus, that is, the structure described by Schaudinn and by Guilliermond.

In the group of spirilla, Dobell notices these three types of structure: In some species he finds present a spherical body resembling a nucleus; other species show a zigzag or a spiral filament; still others have a diffuse nucleus.

From these observations, Dobell feels authorized to conclude that bacteria are organisms originally containing a nucleus, but in which the nucleus, as a result of parasitism, has undergone a series of retrogressions which have ended by making it diffuse.

This opinion would have the advantage of reconciling opposed theories. It would explain how some authors have been able to discern a true nucleus in various forms.

Another more weighty reasoning which might also explain these contradictions is the fact that under the name of bacteria are gathered forms perhaps very different, some of which seem to belong to the *Sulfo-bacteria* and others might be considered as molds.

Although we have just mentioned numerous works, the conclusion, to my mind, would be that while some bacteria may contain a more or less rudimentary nucleus whose existence is nowhere else precisely demonstrated, so far, in the great majority of the species, nothing more has been found than a diffuse nucleus consisting only of grains of chromatin scattered through the cytoplasm.

Life Cycle of Bacteria.—The Editor feels justified in adding to the foregoing review the very recent work of Löhnis and Smith, *Journal of Agricultural Research,* Vol. VI, No. 18, July 31, 1916, because both for its suggestiveness and presentation of experimental

evidence, it can not be disregarded in the intimate study of bacterial cells. Below is given in full the summary by the authors.

Summary by Authors.—A comparative study of 42 strains of bacteria has shown that the life cycles of these organisms are not less complicated than those of other micro-organisms. As representatives of practically all groups of bacteria have been tested and all, without exception, behaved essentially in the same manner, in all probability analogous results may be expected with all species of bacteria.

"All bacteria studied live alternately in an organized and in an amorphous stage. The latter has been called the "symplastic" stage, because at this time the living matter previously inclosed in the separate cells undergoes a thorough mixing either by a complete disintegration of cell wall, as well as cell content, or by a "melting together" of the content of many cells which leave their empty cell walls behind them. In the first case a readily stainable, in the latter case an unstainable 'symplasm' is produced.

"According to the different formation and quality of the symplasm the development of new individual cells from this stage follows various lines. In all cases at first "regenerative units" become visible. These increase in size, turning into "regenerative bodies," which later, either by germinating or by stretching, become cells of normal shape. In some cases the regenerative bodies also return temporarily into the symplastic stage.

"Besides the formation of the symplasm, another mode of interaction between the plasmatic substances in bacterial cells has been observed, consisting of the direct union of two or more individual cells. This "conjunction" seems to be of no less general occurrence than the process first mentioned. The physiological significance remains to be studied.

"All bacteria multiply not only by fission but also by the formation of 'gonidia;' these usually become first regenerative bodies, or occasionally exospores. Sometimes the gonidia grow directly to full-sized cells. They, too, can enter the symplastic stage. The gonidia are either liberated by partial or complete dissolution of the cell wall or they develop while still united with their mother cell. In the latter case the cell wall either remains intact or it is pierced by the growing gonidia, which become either buds or branches.

"Some of the gonidia are filterable. They also produce new bacteria either directly or after having entered the symplastic stage.

"The life cycle of each species of bacteria studied is composed of several subcycles showing wide morphological and physiological differences. They are connected with each other by the symplastic stage. Direct changes from one subcycle into another occur, but they are rather rare exceptions. The transformation of spore-free into spore-forming bacteria seems to be dependent on the conditions acting upon the symplasm and regenerative bodies.

"The discovery of the full life cycles of bacteria may be helpful in many directions. Systematic bacteriology now can be established on a firm experimental basis. Physiological studies will win considerably in conformity and accuracy when connected with morphological investigations along these new lines. Several problems in general biology are brought under more promising aspects. Agricultural bacteriology and medical also will derive much benefit."

FIG. 76.—Various bacteria stained by a method which differentiates only the metachromatic corpuscles. 1–4, *Bacillus radicosus*. 5–6, *Bacillus asterosporus*. 7, The same. The cells have formed their spore and the metachromatic corpuscles outside of the spores have not yet been absorbed by it. 8–9, *Spirillum volutans*. 10–11, *Bacillus alvei*.

RESERVE PRODUCTS.*—Besides the grains of chromatin which we have just been considering in bacteria are found other granulations which do not show the characteristics of chromatin and which act as products of nutrition. These granulations are characterized by the reddish color which they assume with most of the aniline blue or violet dyes, as well as with hematoxylin. These bodies, which are common to the majority of the *Protista*, are metachromatic corpuscles.

They are found in larger or smaller numbers according to the species, the age of the cells, and the medium in which they are living. Some bacteria contain few metachromatic corpuscles (*B. radicosus*, *megatherium*, *mycoides*); others produce many (*B. alvei*, *asterosporus*, *Sp. volutans*, *Bact. tuberculosis* and *diphtheriæ*). The metachromatic corpuscles appear at the beginning of development in the form of very

*Prepared by A. Guilliermond.

small grains, which generally increase gradually in size during development, and finally are absorbed in the very old cells. They are sometimes distributed through the whole cell (*Spirillum volutans*) as grains of chromatin (Fig. 76, 8 and 9), but most often they tend to gather at the two poles of the cell, or line up all along the bacillus (Fig. 76, 1 to 4, 6, 10, 11). In some species (*B. alvei, asterosporus, Bact. tuberculosis* and *diphtheriæ*), these corpuscles grow bigger until they attain relatively large dimensions, surpassing the bacillus in size. Thus they cause a series of swellings all along the bacillus, which in consequence appears somewhat like a necklace (Fig. 76, 11). They then give the illusion of spores; one can easily understand the error of some authors who have confused them with spores, notably in the case of the *Bact. tuberculosis*.

In *B. asterosporus*, the metachromatic corpuscles usually appear in the youngest cells, singly and in the shape of a small central granule closely resembling a nucleus and which A. Meyer seems to have taken for such (Fig. 76, 5).

During sporulation, the metachromatic corpuscles exist just outside of the spore (Fig. 76, 7), then are finally absorbed by it. They therefore act like reserve products.

Moreover, in the cells of bacteria other reserve products, notably globules of fat and of glycogen, have been found.

Bacterial Cell Wall.—*General Structure.**—All the bacteria have cell walls and it is these that give definite form to the cell. These walls are rigid and elastic and are probably made up of two layers, the outer one of which is able to deliquesce and form capsules, or perhaps zoogloœa. The inner part retains the elasticity and gives the form to the bacteria. These cell walls are readily permeable to water and it is through them that all of the nourishment of the cell is obtained; that is, there are no openings for the entrance of food or the discharge of by-products, but the intake and output goes on through the cell wall which is entire.

Minute Structure of Cell Wall.†—In some species of large size, the membrane can be distinguished when strongly magnified, and appears with a double contour. Usually it is scarcely visible, and can be observed only when the contents of the cell has been contracted by

* Prepared by W. D. Frost.
† Prepared by A. Guilliermond.

plasmolysis or by a suitable reagent. It is sometimes thin, sometimes more or less thick. In the latter case, it is often possible to recognize two layers, an *inner or cuticular layer*, very thin and transparent; and the other external, not so well defined and thicker, jelly-like in appearance. This latter or *gelatinous layer* seems to result from a special differentiation of the peripheral zones of the inner layer. The outer layer ordinarily resists staining reagents and appears as a kind of transparent zone about the colored elements. It can acquire a relatively great thickness, and the formations described as *capsules* are only an exaggeration of this gelatinous layer.

Schaudinn has been able to observe quite carefully the construction of the cuticular layer in *B. bütschlii*. According to him, the membrane seen in profile would appear to consist of a series of disks alternately clear and cloudy (Fig. 77, *A* and *B*). Seen from the front, it would give the impression of a network whose meshes are more refringent and stain more highly (*C*). It is laid on a peripheral zone of cytoplasm, a kind of ectoplasm with closer network, and is clearly differentiated from the rest of the cytoplasm. The spore is provided with a double membrane and has at one of its poles a sort of micropyle through which germination is effected (Fig. 71, 15 and 16).

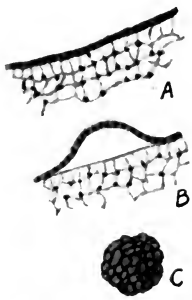


FIG. 77.—*A* and *B*, Structure of the membrane and of the ectoderm in *Bacillus bütschlii*. *C*, Membrane of the same bacillus, front view. (*After Schaudinn.*)

The chemical composition of the membrane is little known. According to some authors, this membrane consists of cellulose; according to others, it contains a lipoid substance; finally, by many authors it is supposed to be composed principally of nitrogenous compounds. Let us remark further that chitin has supposedly been detected therein.

*Capsules.**—A considerable number of the bacteria regularly, or under certain conditions, form what are known as capsules (Fig. 78). These are mucilaginous envelopes which in width frequently exceed that of the organism itself. In microscopical preparations of bacteria it is important to differentiate these from artifacts, since by ordinary staining methods the capsules are not colored but appear as colorless

* Prepared by W. D. Frost.

areas surrounding the bacteria. If, due to shrinkage of the bacteria, or other material on the preparation, clear spaces are formed, it is readily seen that these might be confused with the real capsule. It is possible to stain the capsules by special methods; these must be used in order to determine positively the existence of the capsules. The bacteria which grow in the bodies of animals frequently contain these capsules but fail to show them when grown upon artificial culture media. It is difficult, therefore, to determine whether or not an organism has a capsule by mere examination of cultures. Some culture media, how-

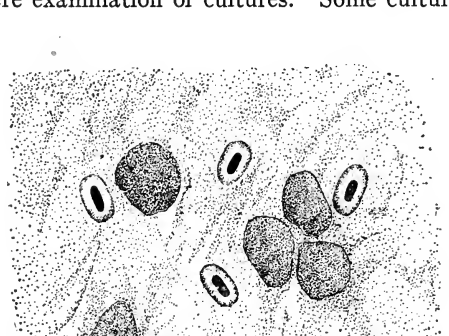


FIG. 78.—Capsules. *Bact. pneumoniæ* (Friedlander). (*After Weichselbaum from Frost and McCampbell.*)

ever, do cause a formation of capsules in the case of capsulated bacteria. These are blood serum, sometimes, and milk, usually. Beautiful capsules can be obtained by growing such bacteria as the *Bact. pneumoniæ*, *Bact. capsulatum*, and *Bact. welchii* in milk cultures. *Strept. mesenteroides* is a bacterium which grows in the syrup of the sugar refineries and forms abundant capsules. This organism changes the character of the syrup, and its entrance and growth is frequently the cause of serious loss.

FLAGELLA.—*General Consideration of Flagella.**—The flagella are very narrow thread-like structures. It is not known how narrow since

* Prepared by W. D. Frost.

they cannot be seen without staining and they can only be stained by precipitating some chemical which may add considerably to their width. They are frequently longer than the organism which possesses

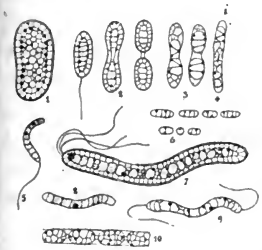


FIG. 79.

FIG. 80.

FIG. 81.

FIG. 79.—*Chromatium okenii;* 2, *Bacterium lineola;* 3, 4 and 5, sulpho-bacteria; 7, *Ophidomonas jenensis;* 8, and 9, *Spirillum undula;* 10, *Cladothrix dichotoma.* (*After Bütschli from Guilliermond review, Bull. Inst. Past.*)

FIG. 80.—*Microspira comma.* Monotrichous bacteria. (*After Migula from Schmidt and Weiss.*)

FIG. 81.—*Pseudomonas pyocyanea.* Monotrichous bacteria. (*After Migula from Schmidt and Weiss.*)

them and sometimes many times that length. *B. symptomatici anthracis* found in the soil has a flagellum sixty times its own length. The arrangement of the flagella on the bacteria is quite constant and

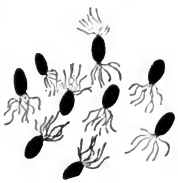


FIG. 82.

FIG. 83.

FIG. 84.

FIG. 82.—*Pseudomonas syncyanea.* Lophotrichous bacteria. (*After Migula from Schmidt and Weiss.*)

FIG. 83.—*Spirillum rubrum.* Lophotrichous bacteria. (*After Migula from Schmidt and Weiss.*)

FIG. 84.—*Bacillus typhosus.* Peritrichous bacteria. (*After Migula from Schmidt and Weiss, and Frost and McCampbell.*)

is used by some authors to differentiate genera. Very few of the micrococci are provided with flagella, as was indicated above, and in the bacilli and spirilla they may be arranged at the poles singly or in

brushes, or they may be arranged on the entire periphery of the cells. When bacteria are provided with a single flagellum at one pole, the arrangement is said to be *monotrichous* (Figs. 79, 80 and 81). When they are arranged in brushes, the arrangement is spoken of as *lophotrichous* (Figs. 82 and 83) and when they are arranged on the entire periphery, the arrangement is said to be *peritrichous* (Fig. 84). It frequently happens that in the case of the monotrichous and lophotrichous the flagella occur at both ends of the organism. This is explained by the fact that the organism is just undergoing binary fission and that the second group is on the newly forming cell. It is worth while in this connection to call attention to the fact that the flagella on one end are new, while those on the other end may be thousands of generations old.

*Minute Consideration of Flagella.**—The question of the cilia or flagella of bacteria is not yet entirely decided. The absence of cilia in large bacteria capable of motion gives the idea that these are not the only organs of motion, and that contraction of the protoplasm certainly plays the most important rôle in the phenomena of motility. Moreover, the nature of cilia has been debated. Van Tieghem and Bütschli, taking their stand primarily on the difficulty of staining cilia by the reagents which rapidly color protoplasm, have considered these cilia to be simply prolongations of the membrane, lacking all contractibility and locomotive power. According to Van Tieghem, when two cells formed by the division of the same element separate, the common portion of the transverse septum, instead of dividing neatly in two, can stretch out into a filament which breaks at a greater or less distance from each of the two daughter cells. This prolongation composes the vibratile cilium.

This theory, however, does not explain the existence in certain bacteria of clusters of cilia at the two poles, or of cilia distributed over the whole surface of the membrane. Other authors, as for example A. Fischer, consider the cilia true prolongations of the protoplasm issuing through tiny apertures in the membrane. This view at present tends more and more to predominate, and the existence of flagella on bacteria appears to be demonstrated.

Another interesting peculiarity, moreover, has recently been established independently by Swellengrebel and by Dangeard. According to these authorities, in some species (*Chromatium okenii* and *Spirillum*

* Prepared by A. Guilliermond.

volutans) the cilia have connection with one of the chromatic grains of the diffuse nucleus. There is a chromatic filament starting from the base of the cilium and ending in connection with a chromatic grain, similar to the organisms with flagella in which the flagellum is in relation to a basal chromatic grain (blepharoplast).

The Higher Bacteria*

The so-called higher bacteria include some of the spiral forms, at least the larger spirochætes, the thread or *trichobacteria*, and the sulphur or *thiobacteria*.

The spirochætes and *trichobacteria* contain so many forms of interest that their form and structure needs special consideration.

The Larger Spirochætes.—Spirochætes differ so much among themselves that it seems necessary to divide them into two groups. The members of one of these groups, the small spirochætes, are practically identical with the true bacteria, and naturally fall in the family of the *Spirilliaceæ*. Members of this group, however, so gradually approach the other group, the large spirochætes, that it is difficult to draw a line of separation between the two, yet the large spirochætes resemble in so many essential details the trypanosomes that they are usually placed as a coördinate genus with them under the flagellates—a sub-class of the *Protozoa*. The larger spirochætes are described as follows:

Form and Size.—In form the spirochætes are long, very thin and flexible spirals. Their length is usually not less than twenty times their breadth. Some forms are as long as 500 μ. It seems probable that some of them are flattened and hence in form are more like a spirally bent ribbon than rod.

Motility.—These organisms move very rapidly under normal conditions. The character of the movement may be of three kinds: (1) Lashing, eel or snake like; (2) undulatory, compared to the flapping of a sail in the wind; (3) rotation, similar to a cork-screw when pushed into a cork.

Reproduction.—Multiplication is by means of binary fission. If these forms are to be considered as bacteria, the division would be expected to be by means of transverse partition walls. A number of workers, however, have described a process of longitudinal division.

*Prepared by W. D. Frost.

Forked forms also which are frequently seen are held to indicate longitudinal divisions. Some observers have claimed that conjugation occurs among the spirochætes. If this is true their relation to the *Protozoa* would be quite likely, but accounts of this phenomenon are inconclusive. Several observers have described "rolled up" specimens, oval and ovoid forms, which have been assumed to be cysts. The spirochætes break up into granules or short segments and such specimens are sometimes spoken of as "monili form." It is not definitely known whether these coccoid forms are simply degenerative forms or the equivalent of bacterial spores.

Sheaths.—A definite sheath has been described for some forms and the irregularity in the disposition of this around the cell may account for the structures that have been taken for undulating membranes.

Cell Aggregates.—There is apparently no definite cell grouping but tangled masses of these organisms have been described in several species.

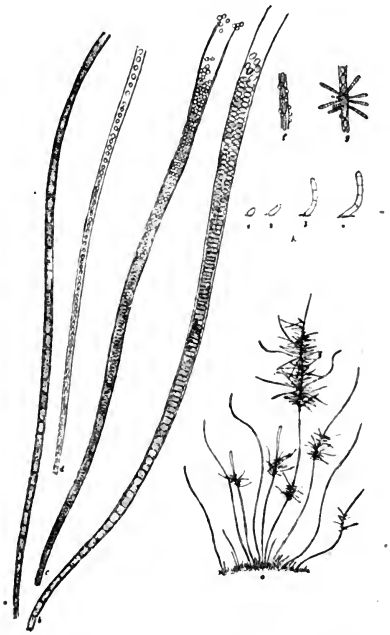
THE TRICHOBACTERIA.—The *trichobacteria* are thread or filamentous forms. The cells are cylindrical and similar in form and may or may not vary in size in different parts of the filament. The individual cells are capable of independent existence, but when growing in the filament give evidence of differentiation in function. Sometimes these filaments are attached to the substratum or some object in it; at other times they are free. In case of the sessile forms the cells at the attached end (base) are smaller than those at the apex. In other members of the group the ends of the thread are swollen or become club-shaped (Figs. 85 and 86). In some forms cell division takes place in three directions of space, thus forming a thread of massed cells.

Branching.—The filaments are usually unbranched, but some forms show true branches, such as is found among the plants—fungi and algæ. Some again exhibit what is called false branching. This is due to a misplaced cell, which grows parallel or at an angle to the parent thread and suggests branching.

Reproduction.—The cells throughout the filament may divide to form spores, but the apical cells of the thread are frequently set apart for the purpose of reproduction, and by a process of division form spores or conidia. The conidia are usually round and without any

esting stage may produce new threads of cells. Sometimes spores erminate while still in the old thread (Fig. 85), giving a tangled nass of cells or whorls of new threads at intervals on the old. The onidia may be either motile or non-motile. The motility of these onidia when it exists is due to flagella.

Sheath.—The threads of cells are sometimes surrounded by sheaths f varying thickness. This sheath is a thickened and hardened mem-



IG. 85.—*Crenothrix polyspora* Cohn, Brunnenfaden. (*After Migula from Schmidt and Weiss.*)

rane, and forms a tube in which the different cells of the bacteria are ontained. This sheath is homologous to a capsule. In it are frequently deposited characteristic by-products of the cell. In *Crenothrix* (an iron bacterium), for example, we have iron oxides.

The best-known member of this group is the water-pest bacterium

(*Crenothrix polyspora*) (Fig. 85), an iron bacterium, which has the power of oxidizing certain forms of iron, causing a deposit to accumulate in the water pipes of cities where it may cause considerable trouble. It is probable also that this bacterium has had a very important part in the deposition of our iron ores, such as those found on the Mesaba range. Another member is the *Actinomyces bovis* (Fig. 153) which is the cause of the common disease in cattle known as lumpy jaw. This bacterium may also infect man. Many other forms of *trichobacteria* are found in nature and probably play important parts in the chemical transformation of matter.

THE SULPHUR BACTERIA.—The sulphur bacteria are filamentous forms which may reach a length of many microns. They are cylindrical or perhaps sometimes flat. They may be either attached or actively motile. The movement when present is due not to flagella, but to an undulatory motion like that of the spirochætes or *Oscillaria* among the algæ. As they move forward they rotate on their own axis and swing their free ends.

Spore formation is unknown in some forms where multiplication is accomplished by the breaking up of the threads in short segments. In the case of the sessile forms conidia are produced at the end of the thread and are motile (*Thiothrix nivea*). The sulphur bacteria contain at certain stages strongly refractile sulphur granules in their bodies.

CLASSIFICATION*

The classification of bacteria was early recognized by Mueller as a matter of difficulty, since he says: "The difficulties that beset the investigation of these microscopic animals are complex; the sure and definite determination (of species) requires so much time, so much of acumen of eye and judgment, so much of perseverance and patience, that there is hardly anything else so difficult." Early investigators found it difficult to decide whether bacteria are plants or animals, and nowadays we are finding it as difficult to decide upon a system of classification. A great many systems have been proposed, but many of them are untenable because those who proposed them were ignorant of or unconcerned by the rules adopted by systematists in other lines. The only system that seems worthy of continued life is that of Migula.

* Prepared by W. D. Frost.

who is a trained botanist. This system, with sight modifications, is given below. In this system, the characters which separate the genera are morphological; while physiological characters, including cultural, are used for the differentiation of species and smaller groups. One of the rules adopted by systematists in other lines is the binomial rule. In the violation of this rule, bacteriologists have been great sinners, and some of the names proposed by Migula and others following his system are quite different from those by which well-known forms have been christened by their discoverers.

CLASSIFICATION OF MIGULA (MODIFIED)

The bacteria are phycochrome-free schizomycetous plants which divide in one, two, or three planes. Reproduction takes place by vegetative multiplication (fission). Resting stages in the form of endospores are produced by many species. Motility is noted in some genera, and this is due to flagella. In Beggiatoa and Spirochæta the organs of locomotion are not definitely known.

I. Order: Eubacteria (true bacteria).

The cells are devoid of any nucleus (Zentralkörper) and free from sulphur and bacteriopurpurin, colorless or faintly colored.

I. Suborder: Haplobacterinæ (lower bacteria).

I. Family: Coccaceæ (Zopf) Mig.

The cells are globular when in a free state, but in the various stages of division appear somewhat elliptical. A few species in this family are motile. Cell division takes place in several directions of space. Frequently the cells remain attached together, and under these conditions usually show some flattening of the cell at the point of junction with the cell next to it.

Genus: Streptococcus Billroth.

The cells are globular and do not possess any organs of locomotion. Cell division takes place in only one plane. Usually the cells remain united together after division, producing chains or diplococcus forms. No endospores have been noted.

Genus: Micrococcus (Hallier) Cohn.

The cells are globular and do not possess any organs of locomotion. Cell division takes place in two planes at right angles. If the cells remain attached together after cell division, merismopedia plates are formed. The plates give the appearance of a regular flat mass of cells. No endospores have been noted in this genus.

Genus: Sarcina Goodsir.

The cells are globular and do not possess any organs of locomotion. Cell division takes place in three planes, all perpendicular to each other. Its cells remain attached after division; cube-like packets are formed. The composition of the medium sometimes prevents this typical cube formation.

Genus: Planococcus Migula.

The cells are globular. Cell division takes place in two planes at right angles

similar to genus Micrococcus. The cells of this genus are motile, possessing one or two long flagella. No endospores are produced in this genus.

Genus: Planosarcina MIGULA.

The cells are globular. Cell division takes place in three planes as in Sarcina. Cells are motile, having only one flagellum on each. Cells usually remain united in twos and in tetrads and seldom form packets as Sarcina.

II. Family: Bacteriaceæ MIGULA.

The cells are cylindrical in shape. They vary in length from short almost spherical bodies to very long rods. Cell division takes place in one direction in a plane

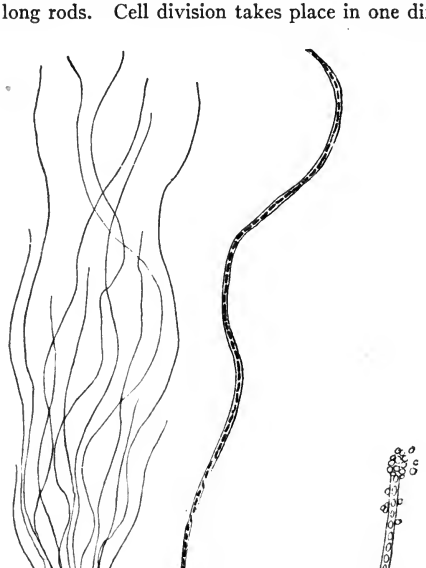


FIG. 86.—*Chamydothrix hyalina* Migula. (*After Migula from Schmidt and Weiss.*)

perpendicular to the long axis of the cell. Some of the members of this family remain attached together, forming threads, while others separate from each other soon after fission.

Genus: Bacterium EHRENBERG.

The cells are cylindrical, of longer or shorter length. Threads are frequently formed. The cells do not possess any organs of locomotion. Endospores are produced in some few species, but in the majority no such formation occurs. It is possible that endospore formation occurs only under certain environmental conditions.

Genus: Bacillus COHN.

The cells are cylindrical, of longer or shorter length. The rods are sometimes oval in shape. Cells are motile and possess flagella which are distributed over the entire surface. Endospore formation occurs with marked regularity. The bacteria in this genus are motile only during certain periods of their life. This period varies greatly in length and occurs only in the vegetative stage.

Genus: Pseudomonas MIGULA.

The cells are cylindrical, of longer or shorter length. The cells are motile and possess polar flagella. These flagella may vary from one to twelve in number. The formation of endospores in this species is claimed by some. If they occur, it is extremely rare. Occasionally certain species in this genus form themselves into threads or chains.

III. Family: Spirillaceæ MIGULA.

The cells are wound in the form of a spiral or representing the portion of a turn of a spiral. In the latter case, if the cells remain attached together in the form of a thread, a full spiral of several turns is produced. Cell division takes place in only one direction of space, and this is transverse to the long axis of the cell.

Genus: Spirosoma MIGULA.

The cells are rigid and bent in the form of spirals. The members of this genus are as a general rule quite large. The cells may be free or united together into small gelatinous masses. Some of the cells individually are surrounded by a gelatinous envelope, while others are free.

Genus: Microspira SCHRÖTER.

The cells are rigid, short, and bent similar to a comma. When the cells are united together, S-shaped threads are formed. The cells are motile, possessing usually one flagellum and rarely two or three flagella. These flagella are about the same length as the cell. No endospores are formed. Some writers make no distinction between Microspira and Spirillum. The name *Vibrio* has also been applied by some writers to this genus.

Genus: Spirillum EHRENBERG.

The cells are rigid, usually long and forming long, screw-like threads, or, in some cases, only portions of a spiral turn. Cells are motile and possess a tuft of flagella at the pole. The flagella may occur at both ends of the spiral, and they vary greatly in number. Endospore formation has been observed in some species.

Genus: Spirochæta EHRENBERG.

The cells are flexible spirals, very thin and long. No flagella are present. These bacteria move by rotation similar to a screw, and also by lateral motion similar to a snake. The locomotive organs, if present, are not known. No endospores are produced.

II. Suborder: Trichobacterinæ (higher bacteria).

Family: Chlamydobacteriaceæ MIGULA.

The cells are cylindrical, are united in threads, and surrounded by a sheath. Reproduction takes place by means of motile and non-motile gonidia. These gonidia arise directly from the vegetative cells and, without any resting stage, produce new threads of cells.

Genus: Chlamydothrix MIGULA.

The cells are cylindrical, non-motile, and arranged in unbranched threads and surrounded by a sheath of varying thickness in different species, being the same diameter at apex and base (Fig. 86). Reproduction takes place by means of gonidia, which are round and arise directly from the vegetative cell. This genus is called Leptothrix by KÜTZING and Streptothrix by COHN.

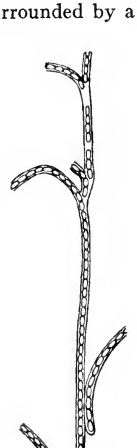


FIG. 87.—*Cladothrix dichotoma* Cohn. (*After Fischer from Schmidt and Weiss.*)

Genus: Crenothrix COHN.

The cells are united together into filaments which are unbranched. The filaments gradually enlarge toward the free end, thus making a distinction between the apex and base. The sheath which covers the filaments is thick and often becomes infiltrated with the hydroxide of iron after being cast off in water in which there is a large amount of iron. Reproduction takes place by the formation of round gonidia which are formed in the beginning by division perpendicular to the long axis of the cell and later by division in three directions of space. Only one or possibly two species can be placed in this genus.

Genus: Phragmidiothrix ENGLER.

The cells in the beginning form unbranched threads. Cell division takes place in three directions of space, thus forming within the sheath a mass of cells. Later these cells may burst through, multiply, and form branches after acquiring sheaths. The sheath in this genus is quite thin and can scarcely be seen.

Genus: Sphærotilus KÜTZING, 1833 (Cladothrix COHN).

The cells are cylindrical and the threads are surrounded by sheaths. Dichotomous branching is present, and there is no differentiation in size between the apex and base of the thread (Fig. 87). Reproduction takes place by means of gonidia which swarm together within the cell. These gonidia burst out of the cells, attach themselves to some object, and grow into new threads. The gonidia are endowed with flagella which are attached toward the end and below the pole.

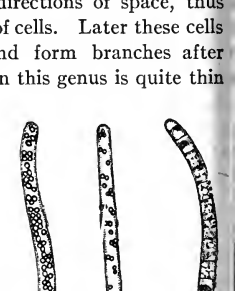


FIG. 88.—*Beggiatoa alba.* Vaucher, Trevisan. (*After Winogradsky from Schmidt and Weiss.*)

II. **Order:** Thiobacteria (sulphur bacteria).

The cells do not possess any nucleus and contain sulphur. The cells are colorless or pigmented rose, violet, or red by bacteriopurpurin. The cells are never pigmented green.

I. Family: Beggiatoaceæ TREVISAN.

Filamentous bacteria which do not contain bacteriopurpurin. The cells contain sulphur granules. Reproduction takes place in one direction of space.

Genus: Thiothrix WINOGRADSKY.

The cells are non-motile and the threads are attached to some object. The threads are surrounded by a delicate sheath and the cells contain sulphur granules. Gonidia are produced at the end of the threads. These gonidia are motile and finally attach themselves to some object, and, according to some authors, bend at right angles in the middle and grow into new threads.

Genus: Beggiatoa TREVISAN.

The threads are not surrounded by a sheath and are formed of flat cells. The cells are not attached (Fig. 88). This genus moves by means of an undulating membrane similar to *Oscillaria*. As the organism moves, it rotates on its long axis and swings its free ends. Gonidia are unknown and reproduction takes place by a division and separation of the threads.

II. **Family:** Rhodobacteriaceæ (WINOGRADSKY's classification, artificial).

The cells contain bacteriopurpurin and on this account may be red, rose, or violet. Sulphur granules may also be included within the cells.

I. **Subfamily.**

The cells are united into colonies. Cell division takes place in three directions of space.

Genus: Thiocystis WINOGRADSKY.

The colonies are small, compact, and enveloped either singly or in groups by a gelatinous cyst. The colonies are also capable of breaking up and the cells moving about.

Genus: Thiocapsa WINOGRADSKY.

The cells are globular in shape and spread out on a substratum in flat colonies. These colonies are surrounded by a common gelatinous secretion similar to a capsule. The cells are non-motile.

Genus: Thiosarcina WINOGRADSKY.

The colonies form packets similar to the genus Sarcina of the *Eubacteria*. The cells are non-motile.

II. **Subfamily** Lamprocystaceæ.

The cells are formed into families. Cell division takes place first in three then in two directions of space.

Genus: Lamprocystis SCHRÖTER.

The cells in the beginning are solid, then hollow, becoming perforated like a net. They separate into small groups and become motile.

III. **Subfamily** Thiopediaceæ.

The cells are united into colonies. Cell division takes place in two directions of space.

Genus: Thiopedia WINOGRADSKY.

The families are formed similar to tubes and are composed of cells arranged in fours and capable of motility.

IV. **Subfamily** Amœbobacteriaceæ.

The cells are united into colonies. Cell division takes place in one direction of space.

Genus: Amœbobacter WINOGRADSKY.

The cells are united into colonies, and after division in one direction of space remain attached together by threads of protoplasm. The colonies possess amœboid motility. The cells change form by contraction and the spreading out of the protoplasm.

Genus: Thiothece WINOGRADSKY.

The colonies are inclosed by a thick, gelatinous cyst. The cells are capable of moving and are very loosely surrounded by a common gelatin.

Genus: Thiodictyon WINOGRADSKY.

The colonies are solid, non-motile, and consist of small cells which are pressed together.

V. **Subfamily** Chromatiaceæ.

The cells are free and capable at all times of motility.

Genus: Chromatium PERTY.

The cells are moderately thick, elliptical or cylindric-elliptical in shape.

Genus: Rhabdochromatium WINOGRADSKY.

The cells are free, rod-shaped, or spindle form; they possess flagella on the poles and are motile at all times.

Genus: Thiospirillum.

The cells are free, continually motile, and spirally twisted.

RELATIONSHIP OF BACTERIA*

There has been a great deal of discussion as to whether bacteria are plants or animals. They were first described as animalcula and to the popular mind they are usually animals or "bugs." It is difficult to determine their exact relation philogenetically. These difficulties are so great that some scientists, as Haeckel, would create a new kingdom, call it *Protista*, and put in it some of the lower plants and animals which are difficult to classify, together with the bacteria. The bacteria are undoubtedly more closely related to the blue-green algæ than to any other forms of life. They resemble these organisms in form, method of reproduction, and absence of definite nucleus. It is quite impossible to decide, furthermore, whether some forms, such as *Bact. viride* and *Bact. chlorinum*, are blue-green algæ or bacteria. On the other hand, there are some points of resemblance between the bacteria and the protozoa. Spore formation, similar to that among the bacteria, occurs among some of the protozoa. Another point of resemblance is the possession of flagella. Some of the flagellates quite closely resemble the bacteria in many ways, and the *Spirochætæ*, which are usually

* Prepared by W. D. Frost.

lieved to be bacteria, have been classed as flagellates by eminent otozoölogists.

Physiologically the bacteria are quite closely related to the fungi, d are frequently classed with them under the term *Schizomycetes*.

Artificial Cultivation of Bacteria*

The introduction of methods of artificial cultivation marks the beginning of the ience of microbiology. These methods were developed by Pasteur and Koch and e depended upon by the microbiologist of to-day as the foundation for most of his ork. It has been the aim of investigation to discover a more general culture edium. So far it has been impossible to do this, but beef broth, made after a rmula suggested by Loeffler many years ago, forms the basis of nearly all of our lture media. This beef broth, or nutrient bouillon, is made by extracting meat ee from fat in water, adding a small per cent of peptone, correcting the chemical action, clarifying and sterilizing. To this broth various substances are added r special purposes; gelatin and agar, in order to solidify the media, and various gars and other chemical substances for the purpose of determining the physiological aracteristics of various bacteria. One of the difficulties with the present methods the artificial cultivation of bacteria is the inconstancy of the composition of the edia, due to the fact that the extract of beef, the peptone, and other ingredients, nnot be obtained chemically pure. If it should prove possible to use synthetic bstances, such as the polypeptids, it would mark a great step in advance, but it is obably quite impossible to devise a single medium upon which all bacteria will ow. Some bacteria, such as those which produce nitrification, refuse to grow on rdinary media containing organic material. The cultivation of bacteria in pure lture is dependent upon isolation, and the method of isolation suggested by Robert och in 1880, and known as the plate culture method, has given eminent satisction. This method is dependent upon the use of liquefiable solid media, such s gelatin or agar.

*Prepared by W. D. Frost.

CHAPTER V

INVISIBLE MICROÖRGANISMS*

The term "invisible microörganism" is used interchangeably with such expressions as "ultra-microscopic organism," "invisible virus" and "filterable virus" to designate a group of microörganisms which for the most part, cannot be discerned with the most powerful lenses. Besides being invisible, these microörganisms will pass through the ordinary "bacteria-proof" filters and with one exception,† they have resisted all attempts at cultivation outside of the animal body.

The virus of foot-and-mouth disease may be taken as a typical example. In this disease vesicles form in the mouths and on the feet of infected cattle. The virus is known to be present in the lymph which forms in these vesicles because this lymph will produce typical attacks of foot-and-mouth disease when inoculated into susceptible animals. If now this infectious lymph be diluted with water and passed through a Berkefeld filter the resulting filtrate will be found to be free from all visible microörganisms and in addition the usual culture tests will give negative results. Notwithstanding this apparent sterility, however, the filtrate will produce disease in cattle in the same manner as the unfiltered lymph. It is known that the symptoms produced by the filtrate are caused by a living organism and not by a toxin, because by successive filtrations and inoculations the disease can be transmitted through a long series of animals, thus indicating clearly that there exists in the filtered lymph a living organism which is capable of reproduction. Another proof that the virulence of the filtered lymph is caused by the presence of living corpuscular elements, and that it is not a mere solution of a toxin, is found in the failure of the virus to pass through filters of finer grain than the Berkefeld as, for example, the Kitasato filter.

The more important of the diseases which may be caused by invisible microörganisms are yellow fever, infantile paralysis, hog cholera, bovine

*Prepared by M. Dorset.

†Bovine pleuropneumonia and such others as may respond to the cultural method of Noguchi.

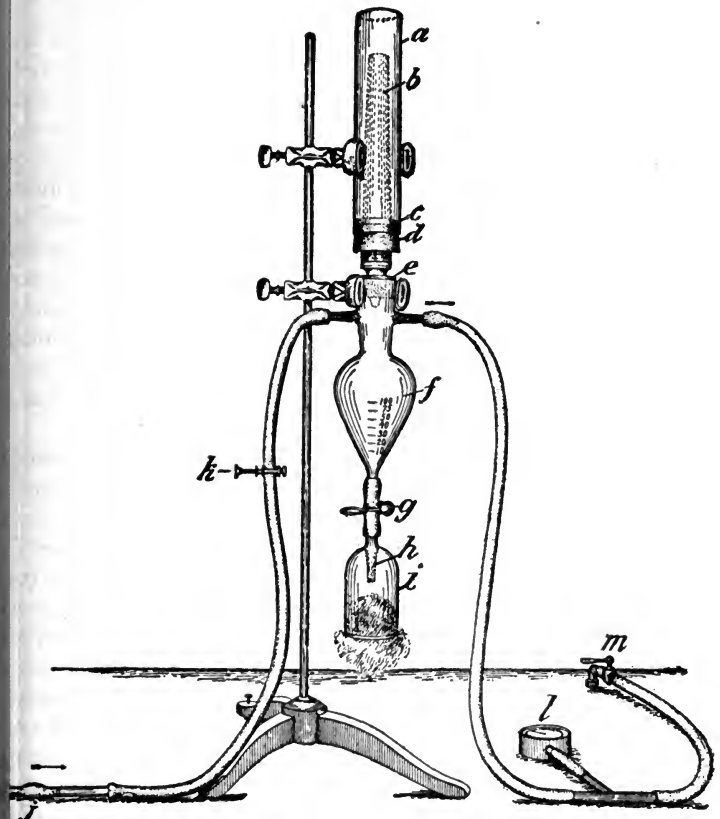


FIG. 89.—Apparatus for fractional filtration, designed for use with Pasteur-Chamberland or Berkefeld filters. *a*, Glass mantle surrounding filter; *b*, Chamberland filter; *c*, paraffin joint; *d* and *e*, rubber stoppers; *f*, double side-arm suction flask; *g* pinchcock controlling outlet from suction flask; *h*, outlet tube surrounded by glass shield and attached to lower end of suction flask by means of short rubber tubing; *i* glass shield fused to and surrounding outlet tube as a protection against contamination when the filtrates are drawn off; *j*, glass inlet tube plugged with cotton, for admitting air into suction flask; *k*, pinchcock governing the admission of air into flask; *l* vacuum gauge; *m*, stopcock connected with vacuum pump. (*U. S. Dept. of Agriculture, Bureau of Animal Industry, Bull.* 113.)

pleuropneumonia, cattle plague, swamp fever or infectious anæmia of horses, chicken pest, sheep pox, and horse sickness.

The invisibility of this group of microörganisms may depend upon either their minute size or their peculiar structure. The most powerful microscopes will not enable us to discern with distinctness objects which are less than 0.1μ in diameter. We know of bacteria which in size approach this limit quite closely (*M. progrediens*, 0.15μ in diameter) and there is no reason for believing that the size of organisms is limited by our ability to see them. As already stated, invisibility may also result from a peculiarity of structure, such as complete transparency and failure to stain with the reagents ordinarily used for this purpose.

The ability of microörganisms to pass through filters is dependent upon a variety of factors. The size and plasticity of the organism, the fineness of the pores, and the thickness of the walls of the filter as well as the conditions under which the filtration is performed, will all influence the result.

The failure of the invisible microörganisms to develop under artificial conditions is to be attributed to their strict parasitism and to our inability to imitate exactly in the laboratory the conditions which exist in the animal body.

While the invisible microörganisms possess certain qualities in common, in some respects they differ widely from one another. Some will pass only through the coarsest of bacteria-proof filters, while others pass readily through the densest filters, thus indicating wide differences in size or in structure. Some are very susceptible to the action of germicidal agents, whereas others are more resistant than the ordinary bacteria. Some produce disease in only one species of animal, while others show little or no limitation in this respect. The diseases produced by these microörganisms likewise differ markedly, some being comparatively benign and local in character, whereas others appear as the most pronounced septicæmias. Some are extremely contagious, while others can be transferred from one animal to another only by means of an intermediate host. In fact these invisible microörganisms seem to differ among themselves quite as widely as do those which are visible to us.

The existence of an invisible microörganism is determined as follows:

The infectious agent must pass through a bacteria-proof filter, which is free from imperfections as shown by tests with visible organisms of small size. Pressure exceeding one atmosphere should not be employed

during filtration. The time of filtration should not exceed one hour. The filtrate should remain free from all visible bacteria as shown by microscopic examination and cultural tests. The filtrate should possess the specific disease-producing qualities of the unfiltered material. Animals infected with the filtrate should yield material which, after filtration, will in its turn possess the attributes of the original unfiltered material. Recent suggestive developments have thrown some light on the possible nature of filterable viruses. The reader is referred to the work of Flexner and Noguchi since 1912, published in the Journal of Experimental Medicine; he is also requested to read the article by Löhnīs and Smith already mentioned on page 97.

CHAPTER VI

PROTOZOA*

Introduction

Many of the diseases which are known to be due to an infecting agent are caused by bacteria; but others are caused by protozoa.

The bacteria belong to the vegetable kingdom. The protozoa are unicellular animals; they are extremely numerous and are very widely distributed in nature, occurring in water, soil, and in the bodies of most animals.

From a zoölogical point of view, the protozoa constitute an important sub-kingdom. It is sometimes difficult to say whether a minute organism is a plant or an animal. For this reason, primitive unicellular organisms are sometimes classified by themselves, as *Protista* (page 114), a kingdom which thus includes not only primitive organisms which have not yet been definitely established in either group but also certain unicellular animals and plants. It appears important, however, to determine as far as possible the genetic relationship of various organisms and, by the study of their physiology and modes of development to differentiate between those which are plant-like and those which are animal-like in character. The protozoa are thus included in the animal kingdom and have been defined as "unicellular animals." They are to be distinguished, on the one hand from primitive forms such as bacteria which lacking differentiation of nucleus and cytoplasm do not conform to the type of structure of true cells, on the other hand, from unicellular organisms of plant-like character such as algæ and fungi which are included with the *Protophyta*.

Many protozoa live in fresh water. Others live in the sea; chalk is formed from the skeletons of myriads of protozoa which once lived in the ocean. While a large proportion of the protozoa are free-living, others are parasitic on animals and plants. Some of the parasitic protozoa are practically harmless and do no apparent injury to the

* Prepared by J. L. Todd. Revised by E. E. Tyzzer.

hosts which support them; others produce severe diseases. Before mentioning those especially which cause disease (see page 822) it will be well to consider the protozoa as a class and to discuss the characters which all have in common.

Structure of the Protozoa

Most protozoa are so small as to be visible only by the aid of the microscope but certain species are visible to the naked eye as individuals,

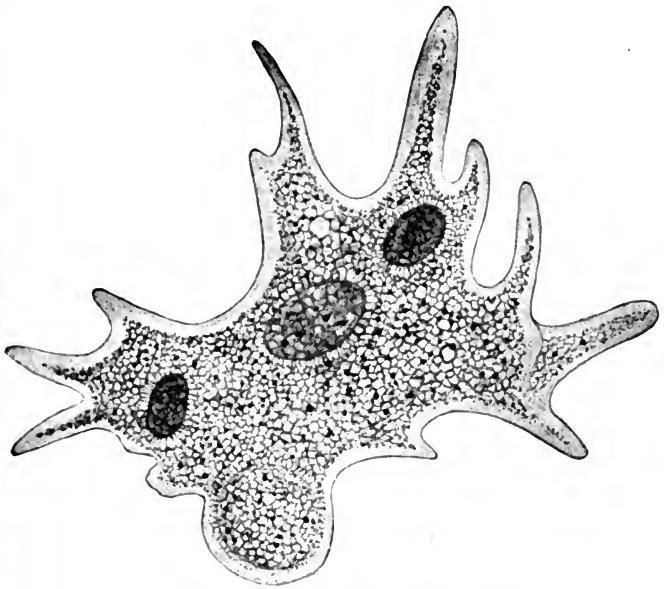


Fig. 90.—*Amœba vespertilio.* (*After Doflein.*)

or as agglomerated masses of individuals. For example, the *Sarcosporidia*, which occur in the muscles of mice and other animals, can easily be seen without a microscope, and the huge plasmodial masses of *Mycetozoa*, which are sometimes seen on rotting wood or in tan pits, may measure many centimeters in breadth.

Like all living things, the protozoa are composed of protoplasm (page 18) and its products. Protoplasm is a complex mixture of various substances in a colloidal condition. When studied by appropriate methods,

the protoplasm of a cell appears to be alveolar or foam-like in structure. This is because the protoplasm is emulsoidal in character being composed of a mixture of many more or less non-miscible substances, some of which are fluid in character, others more of the nature of solids. In such a mixture, the more viscid materials form tiny globules, and each of these is surrounded by a layer of softer material (Fig. 90). The alveolar or foam-like appearance of the cytoplasm of a living cell is somewhat similar to that of bubbles in a mass of foam which is artificially produced. The walls of the outer layer of alveoli, or of alveoli which surround a resistant structure within the cell, are perpendicular to the surface against which they lie, but the outline of the alveoli, which are not in contact with a firm structure, is more nearly circular. An exactly similar arrangement of the alveoli may be seen in a mass of soapsuds contained in a bottle; wherever the bubbles touch an unyielding surface, their outline becomes rectangular.

Recent studies in colloidal chemistry and in the microscopic dissection of cells have furnished valuable contributions to the knowledge of the chemical and physical properties of protoplasm. The view has been advanced that protoplasm consists largely of material in a state known in colloidal chemistry as a *gel*, some portions being firm and viscid and others very soft in character. Procedures which convert such material into a *sol* or fluid state are said to cause the protoplasm to quickly disintegrate. Certain portions of the cell such as the limiting membrane, the nuclear membrane and the nucleolus are of firmer consistence than other portions, and some cells contain globules and granules of various types.

The protoplasm of a protozoön may be divided into two main portions: the *cytoplasm* and the *nucleus*, Chapter I. The cytoplasm, as a whole, may be divided, more or less easily, into a clearer, denser, more resistant outer layer—the *ectoplasm;* and a more fluid, granular, internal portion—the *endoplasm*. Denser, more resistant fibers sometimes run through the cytoplasm and, like a skeleton, serve to fix the shape of the organism in which they exist.

The nucleus, in its simplest form, is a structure which is differentiated from the remainder of the cell by being more refractile and by being colored more deeply in specimens which have been stained by dyes. It stains deeply because it contains a substance called *chromatin*. The chromatin usually occurs in granules which may vary

considerably in size and which are supported upon a *linin* framework that does not stain by ordinary methods. The interstices of the nucleus are filled with nuclear sap. A limiting nuclear membrane may be present, but it is not an essential part of the nucleus. The nuclear material may be all gathered together in a single mass, or it may be distributed in small granules termed *chromidia* so that, at the first glance, no nucleus seems to be present. Such chromidia may be said to constitute a distributed nucleus, although the term nucleus is usually applied to a well differentiated cell structure.

The nucleus (page 15) is to be regarded as the most important unit in the structure of the cell and is apparently essential for the continued existence of the latter. If cells are bisected portions containing no nucleus invariably die while portions containing the nucleus may continue to live and eventually recover from the injury. The rôle of the nucleus is not fully understood but it seems certain that it is a controlling center for the cell's activities. It is concerned in the nutrition of the cell, frequently nuclear structures have to do with the motility of cells and the chromatin serves as a medium for the hereditary transmission of specific characteristics. Its functions, therefore, are at least three-fold since it is active in a trophic, kinetic and reproductive capacities. Usually, all these functions are subserved by a single nucleus; sometimes, however, as in the flagellates and many ciliates they are divided between two nuclei.

Activities of the Protozoa

Whereas the higher animals or *Metazoa* are composed of a great number of cells, a protozoön consists of a single cell. In the former the various functions of the body are each carried out by a special type of cell; for example, movement is performed by the muscle cells, digestion is provided for by the cells of the alimentary tract, and urine is excreted by the kidney cells. A protozoön being a unicellular animal, these various functions must be performed within the single cell of which it consists. Consequently certain parts of its protoplasm are especially differentiated and functionate in a manner similar to the organs of multicellular animals. Such differentiated parts are termed *organellæ* and by means of these the protozoa move about, feed, and excrete waste products in many respects like the higher animals.

The activities of a protozoon may be considered under LOCOMOTION, METABOLISM* and REPRODUCTION.

LOCOMOTION.—The protozoa have several different modes of moving themselves about. Some of them move by the formation of temporary processes or *pseudopodia;* in this method of progression, the protoplasm flows out, in finger-like processes, from the body of the organism and, as the protoplasm flows into these processes, the whole organism progresses, literally, by flowing along. Some of the gregarines move about by means of a flowing of the protoplasm which always takes place in one direction; it is probable that the control of the direction of the flow in these parasites is effected by the contraction of *myonemes.* These are contractile fibers, which usually lie near the surface of the organism possessing them. Through their contraction, the form of the body of the parasite may be altered and, in this way, motion may be produced. *Cilia* are small hair-like processes, which may occur either in definite areas or in large numbers over the whole surface of a protozoon. They produce motion by waving and acting together make a strong simultaneous stroke in one common direction. The movement of all the cilia of an organism is, however, usually not synchronous but proceeds in waves across the surface of its body so that the appearance is similar to that produced when a breeze passes across a field of grain. *Flagella* are larger than cilia; they are whip-like processes which have a lashing movement. They are usually few in number and are often placed at the ends of the organism. *Undulating membranes* consist either of a thin fold of the surface layer or of rows of fused cilia and form either fin-like organs ex-

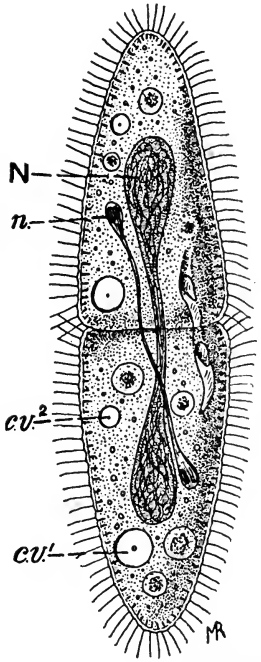


FIG. 91.—*Paramecium caudatum:* division showing the macronucleus (N) dividing without mitosis, the micronucleus (*n*) dividing mitotically. $c.v^1$., Old, and $c.v^2$., new, contractile vacuoles. (*Minchin, after Bütschli and Schewiakoff, in Leuchart and Nitsche's Zoologische Wandtaflen, No.* LXV.)

* Will be treated in Part II, Physiology.

tending along the surface of the organisms or special organs for the intake of food.

Reproduction.—The protozoa reproduce in many different ways and several of these ways may occur in a single organism. For this reason, their reproductive power is very great; in power of repeating their like, they fall just short of the bacteria. The union of a male and a female form does not always precede multiplication; sexual union

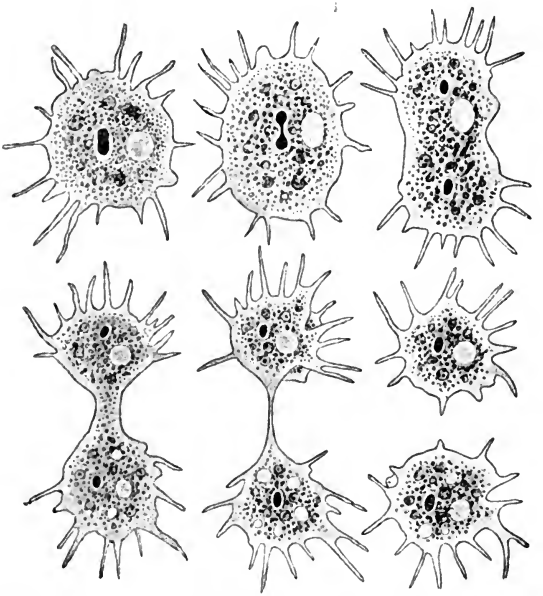


Fig. 92.—Stages in the division of *Amœba polypodia*. (*After F. E. Schulze and Lange from Doflein.*)

and reproduction, though now combined in many animals, may have been originally two entirely distinct phenomena and, in the protozoa, though sexual union may be concerned with the production of new individuals, it is often especially associated with the regeneration of the protoplasm of the parasites taking part in it.

The simplest of the methods of reproduction is simple *binary division*, in which the organism divides into two equal parts. A modification of this process is *gemmulation*, in which a small protozoön buds off

from a larger parent; sometimes many buds are formed rapidly, one after the other, until the parent protozoön disappears in a swarm of daughter cells. When a protozoön divides at a single division to produce a large number of daughter cells simultaneously, the process is

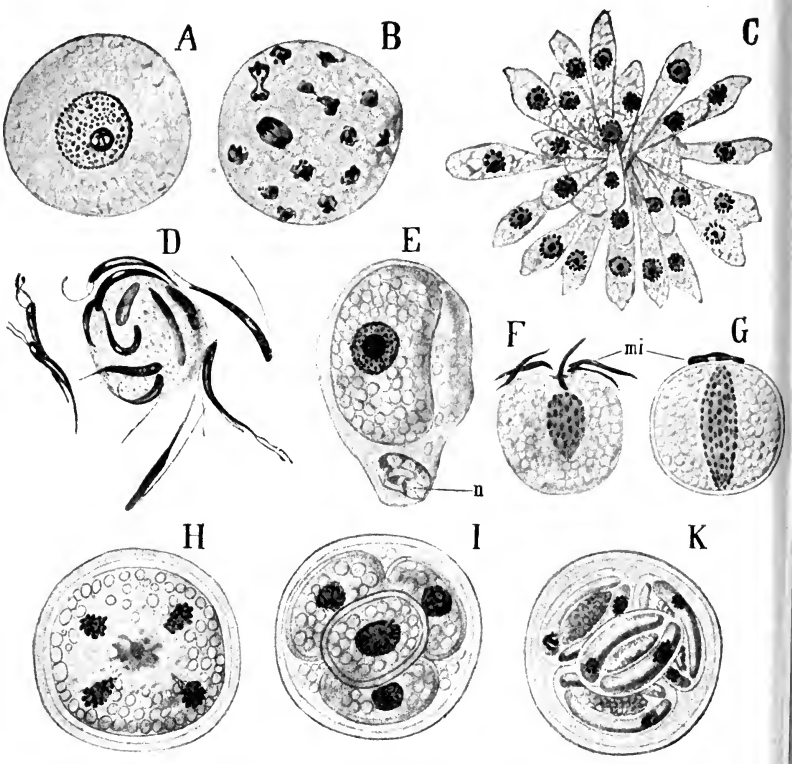


FIG. 93.—*Coccidium schubergi*. *A–C*, asexual multiplication; *D–K*, sexual multiplication; *D*, microgametes; *E*, macrogamete; *F*, *G*, fertilization; *H*, *I*, *K*, division and spore production. (*After Schaudinn, from Doflein.*)

called *schizogony* and the young parasites are called *merozoites*, *i.e.*, if a sexual fertilization has not immediately preceded the act of division; if such a division, in which the parent organism disappears, takes place after a fertilizing act, the process is called *sporogony* and the young parasites are *sporozoites*.

In protozoa, as in metazoa, the essential process in fertilization is the union of two nuclei of opposite sex. In the metazoa the nuclei of the germ cells undergo before they are ready to unite repeated divisions, in which the number of the chromosomes is reduced to one-half the usual number. In dividing, cells may go through a process called *mitosis* during which the chromatin of the nucleus is grouped into more or less rod-shaped masses which are called *chromosomes*. The number of chromosomes which are formed during mitosis is constant and characteristic for each species. In the reproductive areas, during the two divisions just preceding the maturity of cells which are to become ova or spermatozoa, the number of chromosomes is reduced to exactly one-half of the number which are formed during the division of cells outside of the reproductive areas of the same animals. The process by which the number of chromosomes is reduced to one-half is termed *chromatic reduction*, and the fragments of chromatin which in the female are unused and which are extruded from the cell during the process are called *polar bodies*. While reduction in the number of chromosomes has been shown to occur prior to fertilization in a number of the protozoa, in many species a more primitive process consisting of the mere extrusion of masses of chromatin irrespective of the number of chromosomes is found to occur. It is evident that the chromatin is at least usually reduced in amount preparatory to the sexual process.

Although in certain of the protozoa nuclear division is accomplished by a process of mitosis similar to that which occurs in multicellular animals, in many it is affected by a much more primitive process. The nucleus may be resolved into scattered granules of chromatin—chromidia—which may subsequently become reconstructed into a number of nuclei. The nucleus may divide by direct division, that is, by simple constriction into two approximately equal parts. Between this form of division and the classical mitosis there is every possible transition. The centrioles or centrosomes are frequently intranuclear in the protozoa. In case of primitive nuclei without definite nuclear membrane a division simulating mitosis is termed *promitosis*. In other forms in which there is a nuclear membrane but in which the centrioles remain intranuclear throughout division, the process is called *mesomitosis*. The nuclear membrane often persists throughout division and the chromosomes are in many forms very minute or are not definitely formed.

The fertilizing processes which occur in the protozoa may be grouped under three heads: *Copulation, Conjugation* and *Self-fertilization.* In *copulation* two whole cells unite. The cells taking part in this union are called gametes and there are the male or *microgametes,* and the female or *macrogametes.* The cells which produce the gametes are called *gametocytes.* The product of the union is called a *copula* or *zygote.* If the uniting cells be equal in size the copulation is *isogamous;* if they be unequal, the copulation is said to be *anisogamous.* Anisogamous copulation, the union of two unequal cells, is most typically seen in the fertilization of a large macrogamete by a small microgamete. *Copulation* is the most common fertilizing process among the pathogenic protozoa. *Conjugation,* the second method of fertilization, only occurs among the ciliata. In it, two adult individuals place themselves in apposition. The nucleus of each cell first reduces and then divides into two halves, one male, the other female. Each organism retains its female half nucleus, while an exchange of the male half nuclei is effected. Processes of *self-fertilization,* such as *autogamy* and *parthenogenesis,* are included under the third heading. In *autogamy* the nucleus of a single cell divides into two parts. Each of these may undergo further division, during which the chromosomes are reduced or there may be a simple extrusion of a portion of the chromatin. The two resulting, reduced nuclei then unite, in the same cell, to form a new nucleus. *Parthenogenesis* is the development of new individuals from a female cell without a preceding fertilization; this process possibly occurs in many protozoa, and through it perhaps may be explained the reappearance of malaria in patients who once suffered from that disease and were thought to have recovered.

The LIFE CYCLE of a protozoön consists of the changes through which it passes in the period intervening between each fertilizing act. In many of the pathogenic protozoa, an alternation of generations occurs; that is, cycles of development in which an asexual method of reproduction occurs, alternate cycles of development in which reproduction is effected by sexual methods. The developmental cycles are commonly punctuated by binary or multiple division, by encystment, and by transference to a second host as a necessary factor for the completion of the life cycle. An alternation of generations occurs in the life cycle of one of the most important of the pathogenic protozoa, the parasite which produces malaria (Fig. 177). While it is in the body

of its mammalian host, man, it multiplies through multiple fission or schizogony; the sexual, or propagative phase of its development occurs within the body of its invertebrate host, a mosquito. The host in which the adult, sexual stages of the parasite occur, in this instance the mosquito, is said to be the *definitive host;* hosts harboring the parasite while it is in other stages are called *intermediate hosts.*

Encystment.—Under unfavorable conditions, such as dry surroundings, many protozoa are able to surround themselves by a resistant cyst and to enter upon a resting stage of indefinite length. The cyst protects them from harmful influences and, surrounded by it, they remain in a resting state until favorable circumstances come about once more. The power of forming resistant cysts plays an important part in the life history of many parasitic protozoa; it is especially so with those protozoa which have become so specialized that multiplication or continuous existence independent of their appropriate host has become impossible for them. It is often through the formation of cysts that an infection by a protozoön is spread, and, as in the coccidia, the presence of such a stage is often absolutely essential in the life history of a parasite.

Parasitism

A parasite is an organism which is, at some time, directly dependent upon another, usually, a larger organism.

The literal meaning of the term, *i.e.*, eating at the table of another, implies living at the expense of or to the detriment of another.

Although the word parasite is often used as though it referred only to organisms belonging to the animal kingdom, parasites may be either animal or vegetable; bacteria and fungi, which live at the expense of other living beings, are parasites just as the disease-producing protozoa, and the biting insects which transmit them, are temporarily parasites.

Most parasites are simple organisms, low in the scale of life. They nourish themselves without exertion, at the expense of their hosts, and as might be expected, their unemployed organs, such as the sensory locomotory and seizing appendages, by means of which food is usually obtained, gradually disappear; degeneration always occurs in an organism which assumes a parasitic mode of life.

Organisms, such as the malarial parasite, which are wholly de-

pendent for existence upon their hosts, are called *obligatory* parasites; those which are not, such as the infusoria usually found in the stomach of herbivorous animals, are *facultative* parasites. Faculative parasites often feed upon organic material provided by the host, and not upon the host itself; but they are capable of living indefinitely apart from the host.

If an organism is attached to a host, and neither harms nor benefits it, such an organism and its host are said to be *commensals*. For example, the spirochætes found about the teeth of many persons are usually harmless; they are commensals of their host. If the host of an obligatory parasite dies, the parasite may perish also. Consequently, it is contrary to the interest of such a parasite to destroy its host; yet parasites often do harm their hosts. The harm done by a parasite to its host expresses itself in derangements in the physiology of the latter which are known as disease. The pathogenic protozoa may injure their hosts in at least three ways: They may feed upon, and destroy cells; they may produce poisonous toxins; and their presence may do damage by mechanically obstructing some of the functions of its host. All three of these ways are well exemplified by the action of the malarial parasite in man (page 832).

Discussion of the Classification*

The following grouping of the *Protozoa* gives a general idea of the position, in zoölogical sequence, of the individual parasites which are spoken of in the subsequent pages. The *Protozoa* are here grouped into four classes: the RHIZOPODA, the FLAGELLATA, the SPOROZOA, and the INFUSORIA; and these classes are divided directly into genera. This is by no means a complete classification of the protozoan families, for there are many orders, families and genera which are unmentioned because they are parasitic neither in man nor in animals.

The form of a protozoön may vary greatly at different stages of its development; for example, the adult herpetomonas is an active organism moving by means of a flagellum, quite unlike its spherical form which is without a flagellum. Consequently, the whole life history of a protozoön must be known before it can be classified with absolute certainty. The whole of the life history is known for only a few protozoa; and,

*(See p. 13.)

though the organisms mentioned in this classification are placed in the position usually given to them, it must be understood that this classification is not final, and that the discovery of new stages in the life history of some of these protozoa may make it necessary to remove them from the classes in which they have been placed. For example, before its flagellate stage was known, *Leishmania donovani* was classified with the sporozoa; now it is grouped with the herpetomonads.

The characteristics of the different genera and of the unimportant parasites are very briefly mentioned in the following paragraphs; the important parasites are treated more fully in the pages indicated by the references given, in brackets, throughout the classification.

The RHIZOPODA include the simplest forms of animal life. A rhizopod, such as an amœba, consists of a single cell, without a protective covering, and without permanent organs of locomotion; it moves about and captures its food through the agency of its pseudopodia. Very few of the rhizopods are parasitic; most of those which are parasitic, belong to the genus *Entamœba*. Different species of parasitic amœbæ may occur in the alimentary canals of various animals. Certain of these produce serious diseases (page 822).

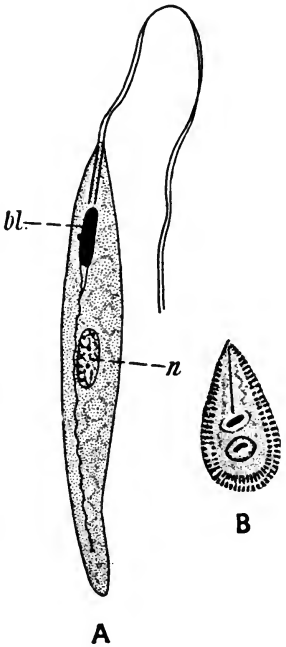


FIG. 94.—*Herpetomonas muscæ-domesticæ* (Burnett). *A*, motile individual with two flagella; *B*, cyst; *n*, nucleus; *bl*, kineotonucleus. (*After Prowazek from Minchin.*)

The FLAGELLATA are distinguished by possessing one or more flagella; they often have, also, a fin-like, undulating membrane extending along the surface of their body. Many possess two nuclei, a larger *trophonucleus* which has to do with nutrition and a smaller kinetonucleus which is intimately connected with the organs of locomotion. This group has thus been termed the *Binucleata* by certain systematists. Most flagellates are free-living. Comparatively few

species are parasitic, but some of these cause very serious diseases (page 824).

The *Herpetomonad* is an elongated organism which possesses *trophonucleus* and *kinetonucleus*. The latter is situated near the flagellar or anterior end of the parasite, and from it arises a terminal flagellum. *Crithidia* is an organism very much resembling an *Herpetomonas*, with a pear-shaped body, and, sometimes, a rudimentary undulating membrane, *Trypanosoma* is an elongated parasite which has a trophonucleus, a kinetonucleus usually situated near its aflagellar extremity and an

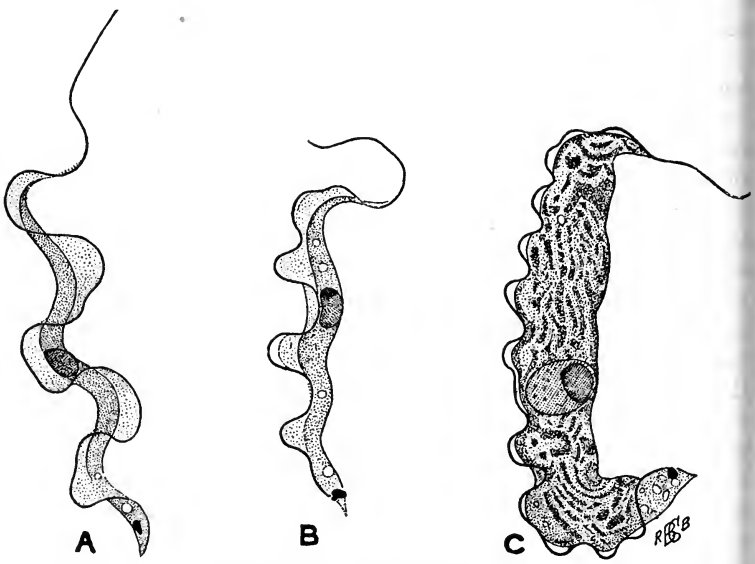


FIG. 95.—*A*, *Trypanosoma tincæ* of the tench; note the very broad and undulating membrane in this species; *B.*, *C.*, *T. percæ* of the perch, slender and stout forms. (*After Minchin*, × 2000.)

undulating membrane along the border of which the flagellum extends to terminate in a whip-like appendage. Species of *Herpetomonas*, *Crithidia* and *Trypanosoma* are frequently found in the intestines of insects. One species of *Herpetomonas* is a frequent and harmless parasite in the intestine of the house fly. The genus *Trypanoplasma* includes organisms which have a flagellum at either end, as well as an undulating membrane. They are parasitic in the blood of fishes. The genera *Cercomonas*, *Monas*, and *Plagiomonas* include small, unimpor-

tant flagellate organisms which have been found, occasionally in man in the alimentary tract, and in necrotic material from the lungs. *Trichomonas* is a pear-shaped organism which has four flagella attached to its blunt end, and an undulating membrane extending from the origin of the flagella at the anterior end posteriorly over the surface of its body. One of the four flagella is usually directed backwards and extends along the border of the undulating membrane.

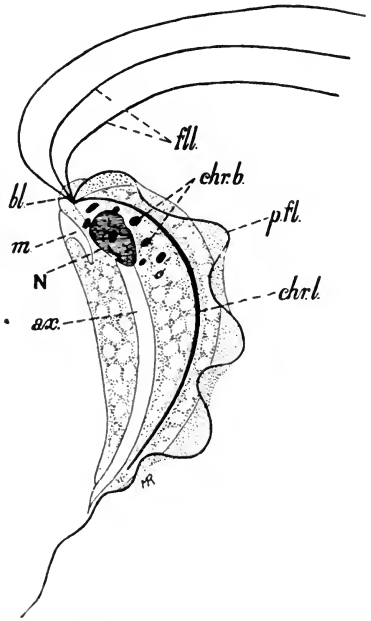


FIG. 96.—*Trichomonas eberthi*, from the intestine of the common fowl; *fll.*, anterior flagella, three in number; *P.fl.*, posterior flagellum, forming the edge of the undulating membrane; *chr. l.*, "chromatinic line," forming the base of the undulating membrane; *chr.b.*, "chromatinic blocks;" *bl.*, blepharoplast from which all four flagella arise; *m.*, mouth opening; *N.*, nucleus; *ax.*, axostyle. (*From Minchin, after Martin and Robertson.*)

One species is sometimes found in the human bladder. Other species are common, usually harmless, parasites in the intestines of pigs, frogs and other animals. The most important species of the genus *Lamblia* is *Lamblia intestinalis*. It also is a pear-shaped organism. It has several flagella and is distinguished by possessing a depressed

sucker, by which it attaches itself to the intestinal epithelium of the animal in which it lives. It is said to cause diarrhœa in man, and also a fatal disease of the intestines in rabbits; but it is almost invariably found in the duodenum and first portion of the small intestine of normal laboratory animals such as mice, rats, and rabbits.

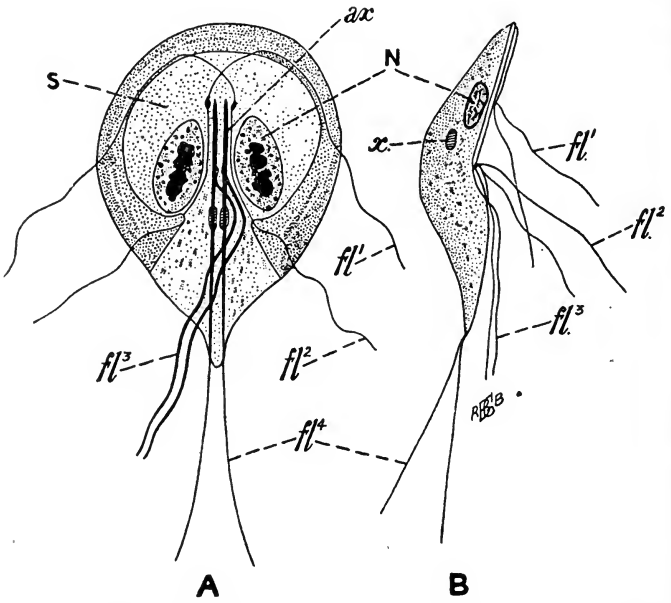


FIG. 97.—*Lamblia intestinalis*. *A*, Ventral view; *N*., one of the two nuclei; *ax*., axostyles; *fl*.¹, *fl*.², *fl*.³, *fl*.⁴, the four pairs of flagella; *s*., sucker-like depressed area on the ventral surface; *x*., bodies of unknown function. (*After Wenyon* (277) *from Minchin*.)

The SPOROZOA are parasitic protozoa which multiply by the production of spores at some stage of their life cycle. There are very many sporozoa and so, for convenience of classification, they are subdivided into seven orders. The *Gregarinæ* have a very distinctive shape; the single cell, of which they are composed, is divided into two or more divisions. The first of these divisions is furnished with hooks or other structures through which the parasite attaches itself to its host. None of the gregarines are parasitic on mammals; worms are the hosts for some of them. The *Coccidia* are usually parasitic within certain cells of their

host, for example, *Eimeria stiedæ* (*Coccidium cuniculi*) (page 831) enters the epithelium of the small intestine and of the bile ducts of the rabbit, while *Coccidium avium* enters and destroys the cells lining the

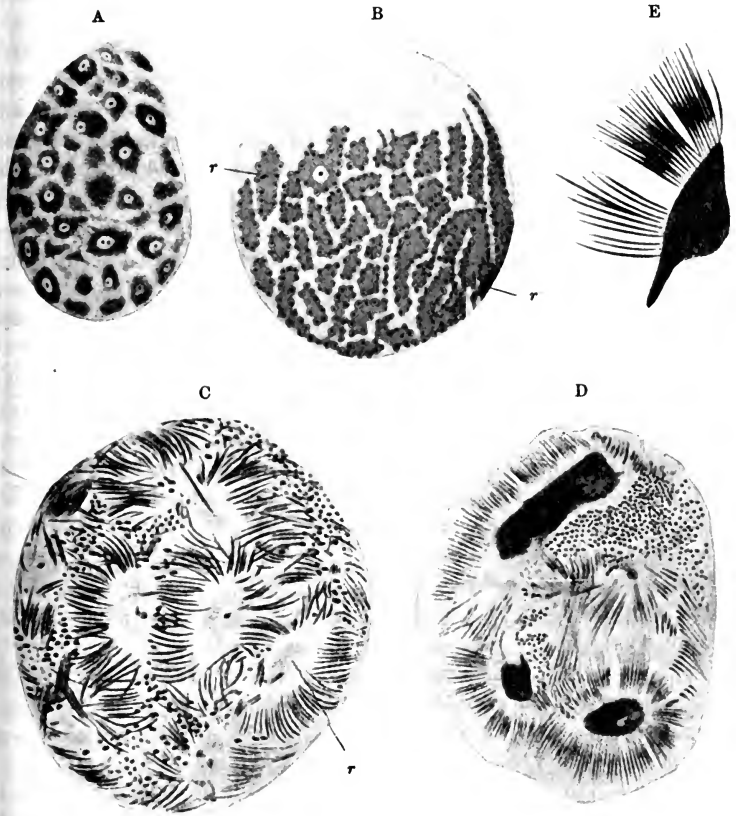


FIG. 98.—Sporozoits in the oocyst of *Laverania malariæ*. *A*, Formation of nuclear points which serve as the foci from which the sporozoits develop; *B*, a more definite shaping of protoplasm and nuclei; *C*, *D*, mature sporozoits in the oocyst arranged about centers from which they radiate; *E*, a portion of one enlarged. (*After Grassi, from Doflein.*)

intestines of the birds which it infects (page 832). The *Hæmosporidia* live, for a part of their life cycle, within the red cells of the blood of

vertebrate animals. They are a very important order. The genus *Plasmodium* causes *malaria in man* (page 832); while *Proteosoma* and *Hæmoproteus* are malarial parasites of birds (page 832). The *Hæmogregarinæ* are usually harmless parasites of reptiles and batrachians (frogs); a part of their life is passed within the red cells of their host, but they have a slowly moving stage, somewhat resembling a gregarine, which occurs free in the blood. *Hepatozoön perniciosum* is the best known of a group of hæmogregarine-like parasites which are parasitic, often within the white cells of the blood, in dogs, in rats, and in other rodents; so far as is known, they do not cause disease. The genus *Babesia* (page 836) includes parasites which cause important diseases in cattle, sheep, horses and dogs. Similar parasites have been found in the blood of monkeys, of dogs, of rats and other rodents. The *Sarcosporidia* are tube-like in shape and filled with spores. They are found within the cells of the voluntary muscles. The *Haplosporidia* are a group of very small sporozoa of which little is known. Some of them are parasitic in fish; one of them, *Rhinosporidium kinealyi*, has been found in a tumor of the nose of a native of India. The *Myxosporidia* (page 841) are recognized by the peculiar form of their spores; each spore has one or more capsules each furnished with a coiled filament or thread which is extruded under certain conditions and probably serves to anchor the spore to a surface upon which further development may occur. Members of this order are parasitic in various tissues of fishes and they often produce disease in their hosts. The spores of the *Microsporidia* (page 841) are exceedingly small; a member of this order is the cause of *pébrine in silk-worms* (page 656).

The INFUSORIA (page 841) are a large class. Most of them are not parasitic. They are the most highly developed of the protozoa and their bodies are more or less covered with cilia, by which they move themselves through the liquids in which they live.

In the last class, under the heading *Parasites of Uncertain Position*, are grouped a number of organisms which cannot be classified because so little is known of them at present. *Histoplasma capsulatum* (page 842), the *Chlamydozoa* (page 842) and the *Ultramicroscopic viruses* (pages 116, 842) are all associated with important diseases in men and in animals.

The SPIROCHÆTÆ (page 843), as their name signifies, are thread-like organisms, which seem to be coiled in a spiral. It is probable that the

curves of certain spirochætes lie in one plane and, consequently, that their bodies are really waved and not spiral. These organisms present no organized nucleus but the chromatin appears to be distributed throughout their bodies.

Those parasites which are important enough to require special consideration are described (page 822) in the order in which they are mentioned in the classification (page 13). Whenever it is possible to do so, a single species is taken as the type of each genus and that species, with the disease it produces, is described; if the remaining species of the genus are mentioned, they are spoken of only to indicate how they differ from the description of the type species.

Technic*

The methods employed in studying the pathogenic protozoa are very similar to those used in bacteriology. Microscopes, with the highest magnifications, are essential for successful work.

It is of great importance in the study of protozoa to examine these organisms in the living condition. In no other way can their mode of locomotion be determined and frequently their contour is also quite different in life and in stained preparations. A small amount of the material in which they occur may be placed beneath a cover-glass on a clean slide and examined immediately with the microscope by ordinary daylight. In case large organisms are examined in rather thin fluid it is well to prevent their being crushed by interposing several minute globules of paraffin between slide and cover-glass which is readily accomplished by touching paraffin with a hot needle and transferring it thus melted to several points on the slide before the preparation is made. When very minute forms are to be studied it is necessary to utilize what is known as the dark field illumination. This brings out very minute organisms and particles which being transparent are invisible to ordinary transmitted light. The dark field apparatus consists of a strong source of light such as a small arc lamp, a special condenser which deflects the light so that objects in the microscopic field are illuminated by light directed from the sides causing them to appear bright on a dark background. Another method of obtaining a dark field is to mix on a slide a small drop of the material to be examined with an equal-sized drop of India ink or better of saturated aqueous solution of nigrosin and then to smear this mixture across the surface of the slide when it may then be dried and examined at once by the oil immersion lens. Only ordinary daylight is required for this method but it does not serve in the study of the motility of organisms.

By special apparatus it is possible after obtaining a certain amount of skill to dissect many forms of protozoa. In this way knowledge is obtained of the physical

* For a more extensive treatise of the technic applicable to the study of protozoa see Doflein, Lehrbuch der Protozoenkunde, Jena, Gustav Fischer; Prowazek, Der mikrochopischen Technik der Protistenuntersuchung, Leipzig; and Stitt, Practical Bacteriology, Bloodwork and Parasitology, Blakiston, Philadelphia.

properties of various portions of their bodies and it is also possible to inject various chemicals into their substance. This method of study is made possible by the mechanical devices utilized by Barbour to whose work the reader is referred.*

In order to make stained preparations the material may be either smeared in a thin film upon clean slides or sectioned after appropriate treatment. In each case the material requires fixation. For the preparation of stained smears the Giemsa method is widely used. This is briefly as follows:

1. Make thin smears of material on a clean slide and dry.
2. Fix immediately by covering the smear with pure methyl alcohol which should be allowed to act for ten to twenty minutes.
3. Dry by waving slide to and fro.
4. Stain for four to twenty-four hours, according to the depth of stain desired, in a solution made by an addition of one drop of Giemsa stain to 1 c.c. of distilled water.
5. Rinse with distilled water.
6. Dry and mount in immersion oil or any acid-free balsam.

It is frequently desirable to keep stained smears unmounted as they apparently retain their color for a longer period of time. They may be studied with the oil immersion lens but the oil should at once be rinsed off with xylol, for if left upon the preparation an insoluble substance is formed which produces a clouded appearance. All stained preparations should be stored away from the light when not in use. For the above method it is important to have all glassware perfectly clean and without trace of acid. The stain must be used immediately after preparation. Certain materials may be smeared very readily with the platinum loop ordinarily used in bacteriology. A very practical method for making blood smears is to gather a minute drop of freshly drawn blood from a small incision or prick in the skin on one edge of the end of a slide. The latter is placed in contact with the surface of another slide and being held at an angle of 45 degrees is pushed steadily lengthwise across its surface. By increasing or decreasing this angle a thicker or thinner film may be made. Certain investigators prefer to use what is termed the wet method for the fixation of smears. In this case the smear is dropped face down immediately and before drying into a fixative composed of two parts of a solution of saturated HgCl in distilled water and one part of absolute alcohol. The technic employed in the staining of sections is then followed and the smear is not allowed to dry at any step in the procedure.

The preparation of stained sections requires a considerable amount of technical skill. Tissue is first fixed to render its structure permanent. It is then dehydrated in alcohol of increasing strengths, next placed in chloroform or some other clearing reagent when it is then imbedded in paraffin after which it may be sectioned. For the details of sectioning and the staining of sections the reader is referred to Mallory and Wright's Pathological Technic, W. B. Saunders and Co., and Lee's Vade mecum.

The cultivation of free-living protozoa is usually accomplished by keeping a supply of the medium in which they live on hand. Hay infusion prepared by boiling

* Barbour: University of Kansas, Science Bulletin 1907–4–3; also Journal of Infectious Diseases, 1911, 8, 248, and 1911, 9, 117.

quantity of chopped hay in water is an easy and valuable method of preparing ulture media. For the cultivation of amœbæ, the following media is widely em-loyed. It should be noted, however, that the amœbæ which have been cultivated re regarded as free-living forms and the attempts to cultivate parasitic amœbæ ave thus far been unsuccessful.

MEDIUM OF MUSGRAVE AND CLEGG

Agar	20 to 30 g.
Liebig's extract of beef	.3 to .5 g.
Common salt	.3 to .5 g.
Water	1,000 c.c.

This medium is designed to provide for slow bacterial growth in order to provide ood for amœbæ. On a richer medium the latter are overwhelmed by the rapid rowth of bacteria.

For the cultivation of trypanosomes, leishmania and other flagellates the so-alled triple N media is employed. This is prepared as follows:

NICOLLE, NOVY, MACNEAL MEDIUM

Water	900 c.c.
Salt	6 g.
Agar	16 g.

issolve, distribute in tubes, sterilize and add to the medium in each tube after quefying and cooling to 40°–50°C. one-third its volume of rabbit blood obtained by ardiac puncture. Slope the tubes for twelve hours, incubate at 37°. for five days o test the sterility of the medium and then keep them at the ordinary temperature f the laboratory for a few days before sowing them. (The tubes should be sealed to revent evaporation.)

The malaria organisms have been made to continue development outside the body y the following method devised by Bass.

Bass's Method.—The blood in 10- to 20-c.c. quantities is taken from the patient's ein and received in a centrifuge tube which contains $\frac{1}{10}$ c.c. of 50 per cent. glucose olution. A glass rod, or piece of tubing, extending to the bottom of the centrifuge ube is used to defibrinate the blood. After centrifugalizing there should be at least inch of serum above the cell sediment. The parasites develop in the upper cell yer about $\frac{1}{50}$ to $\frac{1}{20}$ inch from the top. All of the parasites contained in deeper ing red cells die. To observe the development, red cells from this upper $\frac{1}{20}$-inch ortion are drawn up with a capillary bulb pipette.

Should the cultivation of more than one generation be desired, the leucocyte pper layer must be carefully pipetted off, as the leucocytes immediately destroy the erozoites. Only the parasites within red cells escape phagocytosis. Sexual arasites are much more resistant, and the authors think they observed partheno-enesis. The temperature should be from 40 to 41°. and strict anaerobic conditions bserved. Æstivo-autumnal organisms are more resistant than benign tertian ones. extrose seems to be an essential for the development of the parasites.

PART II

PHYSIOLOGY OF MICROORGANISMS*

DIVISION I

NUTRITION AND METABOLISM

INTRODUCTION

GENERAL PRINCIPLES OF NUTRITION AND METABOLISM.—The nutrition and metabolism of microörganisms are based on the same principles that regulate animal and plant metabolism; in a general way microörganisms are more closely related to animals than to plants, if viewed from the standpoint of their food, their mode of digestion, and their general physiological nature. Only in a few instances, *i.e.*, in the case of life without oxygen (anaerobiosis) and in the ability of some species to use free nitrogen gas, are there processes unparalleled in the more highly developed organisms. Since it will be necessary frequently to refer to plant and animal nutrition in the course of this discussion, these principles, therefore, are briefly discussed in the following paragraph.

Green plants feed only on inorganic substances. They assimilate carbon dioxide (CO_2) from the air which unites with water, nitrates, potassium, calcium, and other salts of the soil and form the body substances of the plant. The cellulose, starch, sugar, protein and all other compounds constituting the plant cells are produced from these simple inorganic substances. This formation of organic compounds from inorganic compounds requires a certain amount of energy. If a certain quantity of sugar is burned to carbon dioxide (CO_2) and to water (H_2O), a certain amount of energy is liberated in the form of heat. The heat given off in this case is also a distinct product of combustion. This heat

*Prepared by Otto Rahn.

is always obtained and always in the same amount regardless of the method chosen in burning the sugar. It has been definitely determined to be 674 calories for 1 g. molecule (180 g.) of sugar. The complete equation of sugar combustion is therefore written

$$C_6H_{12}O_6 + 12O = 6CO_2 + 6H_2O + 674 \text{ Cal.}$$

Consequently the same amount of energy will be needed to produce sugar from carbon dioxide and water; for the law of the conservation of energy requires that, if a certain process liberates a certain quantity of energy, the reverse process will require the same quantity of energy. Green plants get their energy from the sunlight; exactly the opposite proceeds in the equation which should read from right to left; CO_2 and H_2O are absorbed by the plant resulting in the formation of sugar. But it is evident from the equation that CO_2 and H_2O are not sufficient to produce sugar since it takes 674 calories of heat in addition. The radiant energy of light is transformed by the chlorophyl granules of the plant leaves into chemical energy which causes the formation of organic compounds from the simple inorganic or mineral matter. Chlorophyl is the green coloring substance of plants, and only green plants can use the energy of sunlight for their growth.

The growth of green plants is a storing of the energy of light in the form of organic matter; their metabolism is largely synthetic, *i.e.* building up. Plants without chlorophyl, however, like mushrooms, molds, yeasts and bacteria, have to provide for their energy by some other means.

Animals construct their bodies mainly of organic matter. Their body substances as protein, fat, etc., are derived from the protein, fat, cellulose, etc., of plants or of animals. Nevertheless, a certain amount of energy is required in this assimilation process, since the animal protein and fat are somewhat different from the plant protein and fat. Consequently, complex chemical changes and rearrangements which require some energy, are necessary for growth. Energy is also lost by radiation of heat and by locomotion. Animals, being entirely unable to use the sunlight as a source of energy, obtain their energy from the digestion of organic food. The larger part of this food is oxidized completely; this part provides for the energy. The smaller part of the food is used for building the tissues of the body; it becomes part of the animal itself. Animal metabolism is largely analytic, *i.e.*

destructive although a limited amount of energy is required for the chemical changes and molecular rearrangements which are essential to animal tissue formation—a synthetic process. Accordingly more organic matter is decomposed than is formed. Often the same substance can serve both purposes; the meat eaten by a dog furnishes to it energy as well as material for growth. In other cases, certain food compounds execute only one function and not the other. This distinction between food for energy and food for growth must also enter into the interpretation of microbial metabolism.

It might appear from this discussion that energy is needed only by growing cells, as the full-grown cells do not increase in size or weight or number. They also need energy, for in all living cells, there is noticed a continuous breaking down (*katabolism*) and rebuilding (*anabolism*) of the cell constituents. This process is commonly called *metabolism*. The katabolic processes (the breaking down) in a cell will continue even if the cell receives no food. The cell loses in weight, and the starvation which follows will ultimately result in the death of the cell. All living cells require food for the maintenance of life.

In the first part of this book, microörganisms have been divided into plants and animals, but attention has been called in various places to the fact that it is often hard to determine whether the plant characters or the animal characters prevail. This holds true not only with the morphology, but also with the physiology of microörganisms. Since none of the plants discussed in this text-book possesses chlorophyl, none of them can use light as a source of energy, therefore they depend entirely upon chemical energy obtained by the digestion of food. This means that they require organic food almost entirely, since inorganic food furnishes energy only in exceptional cases. In this respect they resemble the animals very much.

The metabolism of protozoa which in some respects calls for differential and special treatment is furnished by Todd and Tyzzer as follows:

"The ingestion of food is accomplished in some protozoa by pseudopodia; the protozoön simply flows around and so encloses a food particle (Fig. 99). In the same way, these protozoa flow away from waste particles which are to be eliminated. Other protozoa have definite mouth areas for the ingestion of food, and definite anal areas for the discharge of residual material. Those protozoa which ingest solid

food, digest it within gastric vacuoles by the aid of enzymes and of acids, just as is the case in many-celled animals. The most important of the disease-producing protozoa live within nutrient fluids, for example the blood, and they obtain their nourishment from the fluid in which they live, by osmosis; consequently, they have no definite mouth area, nor gastric vacuoles.

"Some of the protozoa, for example, some amœbæ and ciliata, possess contractile vacuoles. A contractile vacuole is a clear cavity which

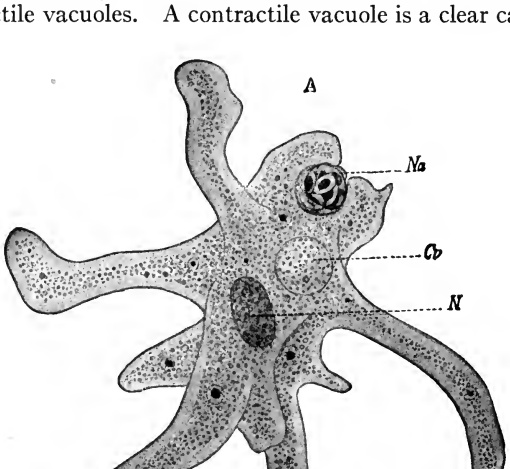


FIG. 99.—*A*, *Amœba proteus; Na*, a food particle; *Cv*, contractile vacuole; *N*, nucleus. (*After Doflein.*)

appears in the cytoplasm, grows slowly, empties itself by a rapid contraction of the fluid which has drained into it and forms again. The fluid which it ejects contains the soluble waste products resulting from the metabolism of the protozoön. One function of the contractile vacuoles is, therefore, excretion; in some protozoa, they are probably also concerned with respiration. Contractile vacuoles are frequently absent in protozoa which are parasitic within other animals.

"Organisms which feed upon solid food, for example the bodies of other organisms, are said to be *holozoic* in their mode of life. Other

hich by reason of their generic relationship are included with the rotozoa are capable under the action of sunlight of manufacturing arch. Such are said to be *holophytic*. Other protozoa live upon rganic material in solution, the *saprozoic* mode of life, and finally me are especially adapted to live at the expense of other animals and re *parasitic* in nature. In the latter instance food may be obtained om the fluids in which they live by absorption as is the case with rypanosomes living in blood plasma or cells may be ingested as in the ase of dysentery amœbæ.

"The process of respiration in the protozoa is in general similar to hat of higher animals. Most of them require oxygen and eliminate arbon dioxide. The contractile vacuole which is found in certain rms is believed to have a respiratory function. Respiration may onsist of the liberation of energy through oxidation or through the reaking down of complex molecules. In organisms of an anaerobic abit the respiration is probably through internal molecular changes ffecting material stored in the cytoplasm.

"In addition to the expulsion of solid undigested material from he cytoplasm there is evidence that waste products other than CO_2 re excreted by contractile vacuoles. Many organisms also secrete naterial either of the nature of chitinous membranes on their surface r metabolic products in the form of globules, etc., within their bodies.

"The most obvious evidence of liberation of energy in the physiology f protozoa is seen in their movement. Certain protozoa, *Nocticula* or example, however, emit light and produce the phosphorescence ften observed in sea water. From analogy with higher animals it is o be supposed that heat and electrical changes are also produced.

"Certain chemical substances which attract protozoa are said to be *positively chemotactic*, others which repel *negatively chemotactic*. Various forms react in a definite manner to light—phototaxis, heat—*thermotoxis*, gravity—*geotaxis*, etc.

"Derangement of function may be produced associated with which re visible degenerative changes. It has also been found that certain rotozoa have the ability to recover from injury and to regenerate lost arts."

Energy Supply of Microörganisms.—The source of energy in microbial life is always of chemical origin. The simplest processes re the oxidations, and simplest among these the inorganic oxidations.

A number of different types feeding exclusively on minerals has been discovered during the last twenty years, and some of them are of great economic importance. They resemble plants in as far as they build their cells exclusively from carbon dioxide, nitrates and ash. The food used for building material is quite different from the food used for the provision of energy.

Two typical examples are the nitrifying organisms in soil which oxidize ammonia to nitrates. This process, according to Winogradski, is divided distinctly into two phases: the *Nitrosomonas* oxidizes the ammonia to nitrous acid,

$$NH_3 + 3O = HNO_2 + H_2O + 78.8 \text{ Cal.}$$

and the *Nitromonas* oxidizes the nitrous acid to nitric acid,

$$HNO_2 + O = HNO_3 + 18.3 \text{ Cal.}$$

These oxidation processes yield a certain amount of energy which enables the bacteria to build their cells from carbon dioxide, ammonia and certain mineral salts. Without ammonia or without nitrous acid respectively, these bacteria cannot grow for lack of energy; they would be like a plant without light. It is evident in this case that the food for energy is also used to some extent as food for growth. The nitrogen necessary to the bacteria is supplied by the ammonia or the nitrous acid.

As an example distinguishing strictly between the food for growth and the food for energy may be mentioned the hyposulphite bacterium studied by Nathanson. This organism oxidizes hyposulphites to sulphates and sulphur, largely following the formula

$$\underset{\text{Hyposulphite}}{Na_2S_2O_3} + O = \underset{\text{Sulphate}}{Na_2SO_4} + \underset{\text{Sulphur}}{S} + x \text{ Cal.}$$

Besides, some more complex compounds, like sodium tetrathionate ($Na_2S_4O_6$), are formed. The bacterium builds its cells exclusively from nitrates, carbon dioxide, and mineral salts; organic food is rejected. The hyposulphite can hardly be used for the construction of the cell and must be considered entirely a food for energy.

This distinction is not confined to mineral decomposition only. The urea bacteria get their energy from the decomposition of urea into ammonium carbonate which is hydrolysis.

$$\underset{\text{Urea}}{(NH_2)_2CO} + 2H_2O = \underset{\substack{\text{Ammonium}\\\text{carbonate}}}{(NH_4)_2CO_3} + 14.3 \text{ Cal.}$$

But the urea and mineral salts are not sufficient for the development of the urea bacteria. They cannot use urea as a material for building the cells, and they cannot use carbon dioxide or carbonates; they cannot grow unless a suitable material for cell construction is added. Söhngen demonstrated that a few milligrams of malic acid favor a good development of the bacteria. The malic acid is used entirely for the formation of cell substances. The energy for this formation came from the urea fermentation. This example shows clearly the different requirements for cell growth and for the energy supply.

With the urea fermentation, we have changed not only from inorganic to organic food, but also from oxidation processes to other decompositions.

Microörganisms differ from the higher animals by their less complete metabolism. The food in the animal, if digested at all, is oxidized as a rule to the final products of combustion, CO_2 and H_2O, the only exception being the nitrogen which leaves the body still in organic combination as urea. With bacteria, yeasts and molds, this is not always the case. Though some of these organisms will bring about complete oxidation of the food we find more commonly incomplete oxidations or changes which require no oxygen at all, but still yield energy to the cell. The biochemical side of these changes of which the alcoholic fermentation is the best known will be discussed in the chapter on oxygen requirements.

CHAPTER I

FOOD OF MICROORGANISMS

The Composition of the Cell

Cells under average conditions may contain certain compounds which are in no way essential to life manifestations; they are in the medium in which the cell grows, and thus pass into the cell without taking part in its functions. Sodium and silicon are probably elements of no use to bacteria though commonly present in the cells. Most of the compounds of the cell are, however, essential to normal development. Some idea of the needs of the cell may be obtained by studying its composition.

Moisture.—The amount of water in the cells of microörganisms will vary with the species as well as with the cultural conditions. The total solids of "mother-of-vinegar" are only 1.7 per cent. This should be considered as an extreme and very unusual case, owing to the spongy nature of the jelly-like cell membrane. The average water content of bacteria seems to be about 85 per cent; it varies more with yeasts and still more with higher fungi. It seems reasonable to suppose that organisms grown in concentrated solutions as the organisms of salted meat and the molds growing in strong sugar solutions contain more solids. Spores of molds contain much more solid matter than the mycelium; the water content in two analyses of spores amounted to about 39 and 44 per cent respectively. Bacterial spores have not been analyzed, but probably are much the same.

Cell Wall.—The membrane of microörganisms does not generally consist of true cellulose $(C_6H_{10}O_5)_x$, though it is found in some cases. Other compounds, related to cellulose, are more common; chitin* $(C_{18}H_{30}N_2O_{12})$, or another very similar nitrogenous compound is also found. The slime surrounding some bacteria, and the capsules consist largely of carbohydrates, but often contain some protein.

* Chitin when hydrolized yields glucosamine and acetic acid.
$C_{18}H_{30}N_2O_{12} + 4H_2O = 2CH_2OH{\cdot}CHOH{\cdot}CHOH{\cdot}CHOH{\cdot}CHNH_2{\cdot}CHO + 3CH_3{\cdot}COOH$

Cell Contents.—The main portion of the cell is the protoplasm, a mixture of protein substances, each of which has a very complex nature. Enzymes which play an important rôle in metabolism (page 178) are produced in the protoplasm and are either secreted or retained. All products of metabolism will be found in the protoplasm of the cell in small quantities. Among other substances frequently found in microörganisms may be mentioned glycogen $(C_6H_{10}O_5)_n$ which can be readily detected by the brown color it gives when acted upon by iodine. Glycogen may be considered as a reserve substance stored by the organism. Another carbohydrate staining blue with iodine is stored by *B. amylobacter*. Fat is commonly found in many bacteria. The amount of fat in some bacteria is surprisingly high. In the tubercle bacterium 26.0 to 39.29 per cent of the total solids is fat. All acid-fast bacterial cells have a very high fat content. Other bacteria also contain occasionally as much as 8 per cent fat. Yeasts seem to have a lower fat content, while in molds it has been found to vary from 0.5 to 50.5 per cent. Many other products of organic nature are found occasionally, but their importance is not determined. Protein is sometimes accumulated in certain places of the cell and gives a granular appearance in the stained cell. Volutin may be such reserve protein.

The minerals of the microbial cell are very essential, and like the organic materials, necessary for the life of the cell. The total ash of bacteria, yeasts, and molds, is small, about 1.5 per cent to 8 per cent of the dry cell. The important minerals which seem necessary for the construction of the cell are potassium, calcium, magnesium, iron, manganese, and of the metalloids, nitrogen, phosphorus, and sulphur. Some other minerals are usually found, but seemingly are unnecessary to the cell, as sodium and silicon.

Amount of Food Required

The amount of food that is ordinarily decomposed by microörganisms and the amount that is absolutely necessary, differ widely. The quantity of organic and inorganic matter just sufficient to support a very weak growth is certainly very small, since a few species will multiply to some extent in ordinary distilled water. Such water, after having stood for some time, is found to contain several thousand bacteria per c.c. It may seem to the layman that in such water it

would be possible to detect easily the organic and inorganic matter of the microörganisms so that it could not be considered distilled water. An estimate of the weight of bacteria demonstrates, however, that this is not the case. If we suppose the average bacterial cell to be a cylinder whose base measures 1 square micron and whose height is 2 microns (which is a high estimate) the volume of such a cell would be $1 \times 1 \times 2$ cubic microns $= 0.001 \times 0.001 \times 0.002$ mm. $= 0.000,000,002$ cu. mm. The specific gravity of bacteria being very nearly 1, the weight of one bacterium would be 0.000,000,002 mg.; 100,000 cells per c.c. means 100,000,000 cells per liter, which would weigh 0.2 mg. Of this total weight, at least four-fifths is water and only one-fifth is solid matter. The total solid matter in 1 liter of water containing 100,000 bacteria per c.c. amounts to the immeasurable quantity of 0.04 mg. Such water will pass the tests for distilled water. How much food the bacteria in distilled water have used is impossible to say, since besides the traces of minerals in the water, they obtain some food from volatile compounds of the air like carbon monoxide (CO), carbon dioxide (CO_2), ammonia (NH_3), hydrogen (H), and perhaps methane (CH_4). Under all circumstances the amount of food used is very small.

On the other extreme, the maximum amount of food cannot be stated very definitely. Usually bacteria cease to cause decomposition because of the accumulation of noxious metabolic products. The ordinary bacterium from sour milk will not form more than about one per cent of lactic acid, because this is the highest acid concentration that this bacterium can endure. If this acid is neutralized, the inhibiting cause is removed, and the lactic fermentation starts anew until the maximum acidity is reached again. The amount of food decomposed depends largely upon the power of the organism to resist its own products. If the food is too concentrated, however, physical influences may interfere with the metabolism of the cell (page 179).

Food for Growth

The total weight of a large bacterial cell is estimated in the preceding paragraph to be about 0.000,000,002 mg., of which only about one-fifth is dry matter. The smallest quantity that can be weighed accurately on ordinary analytical balances is 0.1 mg. This corresponds to about 250,000,000 bacteria. MacNeal and associates found

hat the dry matter of 550,000,000 cells of *B. coli* weigh 0.1 mg. The mount of food that is used as the building material for the cell is robably larger than the weight of the cell itself, since there will always e present waste products, but it is of the same order of magnitude, *i.e.*, ery small and often hardly measurable. The example of the urea fer- nentation (page 146) illustrates this point very well.

Sources of Carbon.—The compounds which can serve as building tones for the cell vary greatly with the species. The source of carbon or all green plants is carbon dioxide, CO_2. Animals cannot use this, or they all require complex compounds, such as carbohydrates, fats r amino-acids. Bacteria exist between the plants and animals in his respect. Some bacteria have already been mentioned (page 147) s being able to use carbon dioxide (CO_2), as the only source of carbon; hey are the mineral-oxidizing species. Such bacteria are called *utotrophic* in their relation to carbon, since they use it in the inorganic orm. A bacterium feeding on carbon, as such, would be called *rototrophic;* bacteria of this class are said to exist. The vast majority of microörganisms are *heterotrophic*, using carbon in organic form. Organic acids and sugars are excellent sources of carbon for micro- rganisms, although proteins and their decomposition products seem o be equally satisfactory as construction material.

Sources of Nitrogen.—The sources of nitrogen are equally varied; he green plants use nitrates; animals must have a number of different mino-acids; the microörganisms again are found between them. We know *autotrophic* bacteria, and especially molds and yeasts which can grow with nitrates or ammonium salts as the only source of nitrogen. There are three groups of *prototrophic* bacteria in their relation to nitrogen—the *B. amylobacter* group, the *Ps. radicicola* group and the *Azotobacter* group. These bacteria are of the greatest importance to agriculture; soil fertility depends, to a large extent, upon the last two groups, for they take nitrogen gas from the surrounding air, form their own protoplasm from it, and thus increase the amount of chemically combined nitrogen in the soil. Details of their relation to soil fertility can be found in Chap. III, page 338. The majority of bacteria are *heterotrophic*, requiring organic nitrogen. Urea is not well adapted for this purpose; amino-acids or the peptones from which amino-acids are derived are the best compounds for most organisms. Asparagin is very commonly used if for some reason peptones are to be omitted.

Sources of Hydrogen and Oxygen.—The sources of hydrogen are hardly ever discussed with bacteria since hydrogen bears such a close and peculiar relation in water and organic food supplies. The ultimate association of hydrogen with oxygen in the molecule of water (H_2O) and with carbon in organic substances (CH_4) establishes its importance in all life processes. There are many *prototrophic* bacteria, using oxygen as such; others are able to reduce such compounds as nitrates or sulphates, which would be *autotrophic*, thus providing for their needs. *Heterotrophic* bacteria are not unusual. In this connection it may be said that it is often difficult to distinguish between oxygen needed for cell construction and oxygen needed for energy formation.

Sources of Minerals.—The amount of mineral matter necessary for the construction of the cell is very small; potassium and phosphorus seem to be among the most essential elements. It is customary to consider a tap water with 0.02 per cent of di-potassium hydrogen phosphate, K_2HPO_4, sufficient in mineral matter of all kinds to provide for fair growth. Some of the common materials used in the preparation of nutrient media, such as meat extract and peptone, also contain considerable amounts of mineral matter.

Food for Energy

As all food in its decomposition results in products of some form or other, it may not seem justifiable to separate a paragraph on *food* from another on *products*. The essential difference lies in the fact that we consider food from the viewpoint of the cell, while products are commonly considered apart from the construction processes of the cell and only from their application, or, it may be, from the viewpoint of usefulness to man.

Animals provide for their energy by oxidations, and almost exclusively by complete oxidations. Some bacteria, and most molds, do the same. The range of materials which can serve as food for this purpose is surprising. With animals, the food is practically limited to plant and animal tissue. With bacteria, we find the strangest substances, such as hydrogen, carbon monoxide, coal, marsh gas, hydrogen sulphide, ammonia, nitrites, formic and oxalic acids, alcohol and thiosulphates serving this purpose. The fact that many gases are used as food makes us realize that oxygen is not such an extraordinary compound as animal physiology seems to indicate, but that it should be

classed merely as one of the many food compounds. This is especially significant since it will be shown later that free oxygen is not necessary for microbial life, and that many organisms can exist without it.

The oxidations are not always complete. The formation of nitrous acid from ammonia, the oxidation of alcohol to acetic acid are such examples. Some organisms are highly specialized in their food requirements, especially the mineral-attacking bacteria are usually limited to one source of energy. The microörganisms oxidizing organic compounds have, as a rule, the ability to decompose several compounds, and some bacteria are common scavengers, able to feed on organic acids, sugars, fats and proteins.

Oxygen Relations

It is characteristic of many microörganisms to provide for their energy without using free oxygen. One such example has already been given in urea fermentation.

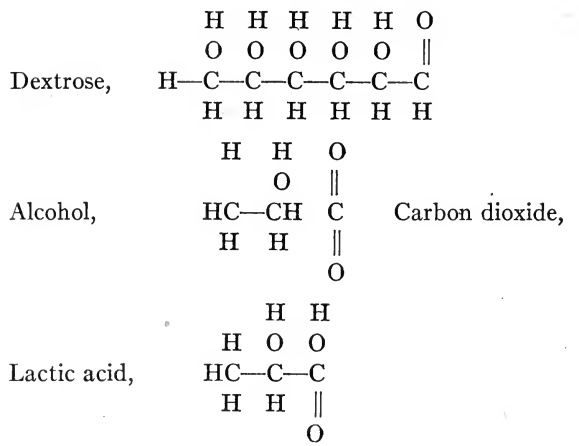
$$\underset{\text{Urea}}{(NH_2)_2CO} + 2H_2O = \underset{\text{Ammonium carbonate}}{(NH_4)_2CO_3}$$

Very common is the decomposition of sugars without oxygen. The two most typical fermentations of this type are the alcoholic and the lactic fermentations.

$$\underset{\text{Sugar}}{C_6H_{12}O_6} = \underset{\text{Alcohol}}{2C_2H_5OH} + 2CO_2 + 22 \text{ Cal.}$$

$$\underset{\text{Sugar}}{C_6H_{12}O_6} = \underset{\text{Lactic acid}}{2C_3H_6O_3} + 15 \text{ Cal.}$$

In fermentations of this type, the changes take place without an oxygen gas partaking in the reactions. These fermentations seem to be essentially reactions of the oxygen atoms within the sugar molecule. One side of the molecule is reduced while the other side is oxidized. In the sugar molecule, each carbon atom has one oxygen atom. In the products of fermentation, carbon dioxide has two oxygen atoms to one carbon atom, and in alcohol there is only one oxygen atom for two carbon atoms. In the lactic fermentation, the oxygen, which is distributed evenly in the sugar, is shifted to one side of the molecule in lactic acid.



In some of the more complex fermentations, we find simultaneous formation of hydrogen or methane and carbon dioxide; the one is the end product of reduction, the other the product of complete oxidation. This also indicates that the oxidation of one part of the molecule takes place at the expense of the other.

In a similar way, some organic acids, *e.g.*, tartaric and lactic acids, can be fermented by certain bacteria without requiring oxygen. Some bacteria have the ability to attack proteins and decompose them completely in the absence of oxygen.

Bacteria, having the ability to provide for their energy without oxygen gas, may live in the complete absence of oxygen, and may multiply indefinitely without it as long as there is sufficient food. But some microörganisms, such as yeasts, seem to grow only for a limited time in the absence of oxygen. Finally, they cease growing, and we may well assume that they need oxygen for cell construction which can be used in no other form except as molecular oxygen. The urea bacteria also belong in this group.

A large number of bacteria and yeasts, and also a few molds, can provide for their energy by either oxidation or decomposition in the absence of oxygen. Very commonly a great variety of compounds can be found which may be oxidized while but very few can be intramolecularly fermented without oxygen. This is easily understood: all organic compounds will yield heat upon oxidation, while exothermic

ıtramolecular changes require a special structure. Carbohydrates re the most excellent substances for such intramolecular decomposi-ions. *S. cerevisiæ* and *B. coli* can live in sugar-free broth only if ex-osed to the air. They provide for all their needs by oxidation of the rotein. If oxygen is excluded, growth depends upon sugar, or a imilar fermentable compound. We test for the absence of sugar in a iven solution by pouring it in a fermentation tube and inoculating ith *B. coli:* if the liquid in the closed arm remains clear, *i.e.*, if *B. coli* oes not grow without oxygen, it is a good indication that no sugar is resent.

It is usually assumed that in fermentations of this nature, the xygen atoms are shifted within the same molecule. In other cases, xygen is taken from one molecule and used for the oxidation of nother. This results in one of the molecules being reduced. Nitrates re reduced in this way to nitrites, or ammonia, or nitrogen gas; sul-hates to hydrogen sulphide, and litmus or methylene blue to the olorless leuco-compounds. Such removal of oxygen from a molecule equires energy, and is possible only when the bacterium by using the xygen for oxidation of organic matter can obtain a larger amount f energy. The following example shows such a possibility:

$$2KNO_3 + 36.6 \text{ Cal.} = 2KNO_2 + O_2$$
$$C_2H_5OH + O_2 = CH_3CO_2H + H_2O + 115 \text{ Cal.}$$

This process leaves an energy balance of $115 - 36.6 = 78.4$ Cal. for he needs of the bacterium.

Such decompositions are sometimes referred to as "reducing fermen-ations" but this term is not correct, as the reduction must always be accompanied by a simultaneous oxidation process.

The amount of energy liberated by a fermentation without oxygen s much smaller than that furnished by complete oxidation; the intra-molecular change always leaves organic compounds which contain a considerable amount of the total energy. Yeast, in presence of very much oxygen, oxidizes sugar completely to water and carbon dioxide.

$$C_6H_{12}O_6 + 12O = 6CO_2 + 6H_2O + 674 \text{ Cal.}$$

while in the absence of oxygen it will change the sugar to alcohol and carbon dioxide.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 22 \text{ Cal.}$$

The energy gained in the first process is about thirty times as large

as that gained in the second process. This was demonstrated as early as 1861 by Pasteur. He grew yeast in sugar solutions, varying only the amount of oxygen in contact with the medium. At the end of the experiment, the weight of the dry yeast and the decomposed sugar was determined, and the amount of sugar necessary to produce one part of yeast was computed. He found:

In a closed flask, without any air.....	1 part yeast required	176 parts sugar.
In a closed flask, with large air space.....	1 part yeast required	23 parts sugar.
In a thin layer, a few mm. thick.....	1 part yeast required	8 parts sugar.
In a very thin layer, in 24 hours.....	1 part yeast required	4 parts sugar.

This experience led Pasteur to the conclusion that fermentation corresponded to the respiration process of animals, that fermentation was respiration without oxygen.

It is quite evident that since the utilization of the food in the absence of oxygen is very high, the organisms have to decompose much more food. This accounts, to a great extent, for the enormous destructive power of bacteria, when comparisons of the great quantity of food decomposed are made with the very insignificant weights of cells. It has been estimated that the lactic bacteria decompose their own weight of sugar in one hour.

Summing up the relation of oxygen to microörganisms, some bacteria, and especially the molds, are found depending upon oxygen as an indispensable part of their food. Three groups are recognized: Those, a large number, organisms in the presence of oxygen producing oxidations; those able to sustain life without oxygen; and those depending entirely upon decompositions which require no oxygen. The lactic bacteria and the butyric bacteria belong in the last group.

In considering the oxygen requirements, it is customary to include another influence of oxygen upon bacteria. This has really nothing to do with its food value, but deals with the poisonous qualities of oxygen. Oxygen in this light may well be called a poison as it will kill bacteria in very low concentrations. Ordinarily it is regarded as constituting over 20 per cent of our atmosphere. But if a study is made of its effect upon bacteria, it is necessary to measure it in the same way food is measured, and consider the concentration in which it is offered to the cell. Microörganisms obtain their oxygen not as gas, but as dissolved oxygen. The solubility of oxygen is very small about 0.0009 per cent at 20°. Practically all bacteria die readily if the

xygen concentration is raised to thirty times the atmospheric pressure. 'his would mean a concentration of 0.027 per cent. It shows that xygen is about as poisonous as formaldehyde or bichloride of mercury.

Some bacteria are extremely sensitive to oxygen, and will die if xposed to ordinary atmospheric oxygen. They grow only if oxygen ; almost completely removed. These organisms are called the *trictly anaerobic* or *obligate anaerobic* bacteria. They are contrasted vith the *facultative anaerobic* bacteria which thrive with oxygen as well s without, and the *strictly aerobic* bacteria which have to have oxygen or their normal life processes.

No strict limits can be drawn between aerobic and anaerobic acteria. Even the most sensitive of organisms will be able to tolerate races of oxygen, while the strictly aerobic bacteria can multiply also f the oxygen concentration is below that of a saturated solution. The imits of growth for the anaerobic bacteria are the limit of tolerance of he poisoning oxygen; the lower limit of growth for the aerobic bacteria s a question of too scanty food supply. The relation between bacteria nd oxygen is graphically represented in the following diagram, after Kruse:

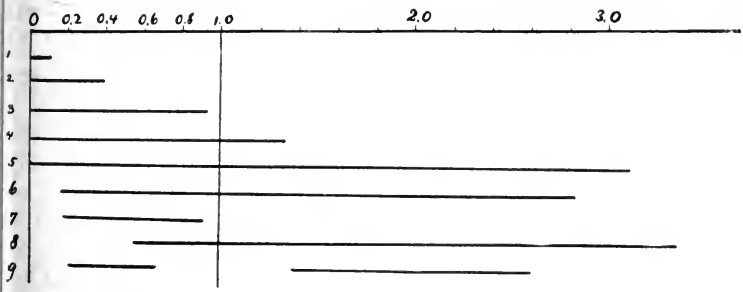


FIG. 100.—Influence of oxygen upon microörganisms.

The lines indicate the oxygen concentrations where growth is possible. Line 1 is a strict anaerobe; 2 is not quite so strict; 3 is still less sensitive though it cannot grow if exposed to direct influence of the atmosphere; 4 is a facultative bacterium such as *B. coli;* 5 is another one which can tolerate still more oxygen; 6 can grow only with oxygen but can get along with very little: it might be one of the urea bacteria; 8 is more dependent upon oxygen and the line would correspond to average molds; 7 is a peculiar type needing oxygen and yet being very sensitive to it. The sulphur bacteria, *e.g.*, the *Beggiatoaceæ*, belong to type 7. Type 9 is said to be representative of *B. abortus*.

CHAPTER II

PRODUCTS OF METABOLISM

General Considerations.—The great difference in the metabolism of animals and of bacteria, even though they feed essentially on the same foods, is the incomplete metabolism of most bacteria contrasting sharply against the very complete oxidation of food in the animal body. The food of the animal is decomposed by the body cells to carbon dioxide, water and urea. It is the most complete decomposition possible, excepting urea which, however, is very near the final decomposition product, ammonium carbonate. Microörganisms, on the contrary, are characterized by incomplete metabolism. They do not commonly oxidize their food to the end products but many of them produce organic compounds which are not farther decomposed by them. It is this partial decomposition of organic matter which makes bacteria play such an important rôle in life and industries. Our modern bacteriology is dated from the time when Pasteur showed that the alcohol in the beer fermentation, the lactic acid in the souring of milk, the acetic acid in the vinegar fermentation are products of microbial activity. The existence of bacteria had been known for nearly 200 years, but they were considered largely as a curiosity as soon as they were recognized as the cause of fermentations, and of toxins, they received at once the greatest attention. Not all bacteria cause incomplete decompositions; some oxidize as completely as animals do. Others, again, form first intermediary products which they later decompose completely; among these, are found many molds, the sulphur bacteria, and some species of the vinegar bacteria.

The Chemical Equations of Fermentations

The metabolism of all organisms is considered to be a chemical process which follows in all respects the laws of chemistry. That we are not familiar with all the changes taking place in the cell is no

because we are dealing with unknown forces, but simply because we do not know all the factors involved in the process. Some of the chemical changes caused by the living cell can be imitated exactly by the chemist in a test-tube. This may be illustrated by the oxidation of alcohol to acetic acid, the decomposition of urea to ammonium carbonate and of ammonia to nitrate. Some other processes are not as fully understood and not as easily imitated. The alcoholic and acid fermentations of sugars are of such nature. There is no reason to suppose, however, that these processes are other than chemical changes. Since a chemical process can always be expressed by a chemical equation, we should expect the same with the fermentations and decompositions caused by microörganisms.

This formulation is not always simple, because the greater number of microörganisms decompose organic substances in more than one way. Also, certain compounds may be produced in such small quantities as to escape the chemical analysis entirely, since the determination of many organic compounds is a very difficult task. Again, part of the decomposed material will usually be assimilated in the growth of the cells; hence more material disappears than can be accounted for by the fermentation products. There are several possibilities for discrepancies; accurate equations can be given only for the simplest fermentations, the products of which can be analyzed more or less exactly.

The best studied microbial process is the alcoholic fermentation. The simplest equation for the decomposition of dextrose into alcohol and carbon dioxide by yeast is

$$\underset{180}{C_6H_{12}O_6} = \underset{92}{2C_2H_5OH} + \underset{88}{2CO_2}$$

According to this formula, 100 parts of dextrose should give 51.11 parts of alcohol and 48.89 parts of carbon dioxide. The actual yield comes very close to these numbers, but does not reach them; the largest amounts found were 46–47.5 per cent of carbon dioxide and 47.5–48.67 per cent of alcohol. Under the most favorable conditions, the total yield of the products of fermentation was only 95 per cent of the theoretical yield.

Other products are formed besides the alcohol and carbon dioxide. The amount of glycerin found in fermented liquids varies very much with the conditions of fermentation; it reaches from 1.6 to 13.8 per cent

of the alcohol or from 0.8 to 6.9 per cent of the fermented sugar. A small quantity of succinic acid is also formed, usually about 0.6 to 0.7 per cent of the fermented sugar. Traces of acetic acid and of lactic acid seem to be normal products of the process of fermentation, and we always find fusel oil. The latest investigations seem to indicate that glycerin and succinic acid are produced by yeast cells even in the absence of sugar. This discovery makes it probable that the glycerin and succinic acid are derived from the reserve substances of the yeast cells, such as lecithin, and are not direct products of fermentation. This accounts also for the variation of the proportion between alcohol and glycerin. Fusel oil is now believed to be a waste product of cell construction.

Similar are the experiences with the lactic fermentation which has been studied almost as extensively as alcoholic fermentation. If it is supposed that the formation of lactic acid follows the equation

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Lactic acid}}{4C_3H_6O_3}$$
$$342 \qquad 18 \qquad 360$$

the actual yield of acid is found to be between 90 per cent and 98 per cent of the theoretical. The other 2–10 per cent are either used for cell-growth or for products which thus far have escaped chemical determination. Small discrepancies will also be found in fermentation of urea and in the nitrifying process, where small amounts of the nitrogenous material are used for cell-growth.

Another difficulty in finding the chemical equation of a microbial fermentation is the fact that this process may change with the age of the culture. In those fermentations where several gases, as carbon dioxide and hydrogen, are produced, the relative proportion of the two is not always constant. In the butyric fermentation of dextrose by *B. amylozyma*, Perdrix tries to account for this change by assuming three different phases of the process at various ages of the cultures, represented by the following equations:

First stage: $\underset{\text{Dextrose}}{56C_6H_{12}O_6} + 42H_2O = 116H_2 + 114CO_2 + 30\underset{\text{Acetic acid}}{CH_3COOH} + 36\underset{\text{Butyric acid}}{CH_3CH_2CH_2COOH}.$

Second stage: $46C_6H_{12}O_6 + 18H_2O = 112H_2 + 94CO_2 + 15CH_3COOH + 38CH_3CH_2CH_2COOH.$

Third stage: $C_6H_{12}O_6 = 2H_2 + 2CO_2 + CH_3CH_2CH_2COOH$.

Kruse has called attention to the fact that these complex equations can well be explained as the simultaneous occurrence of the following simple fermentations:

$$C_6H_{12}O_6 = 2H_2 + 2CO_2 + CH_3CH_2CH_2CO_2H$$
$$C_6H_{12}O_6 = 3CH_3CO_2H$$
$$C_6H_{12}O_6 + 6H_2O = 6CO_2 + 12H_2$$

The first fermentation continues when the others have already ceased, and thus the last stage of Perdrix's equations is very simple. Bredemann also found that the proportion of the various products formed by *B. amylobacter* varies greatly with the conditions, and the same has been recently established in the fermentation of *B. coli*.

Other complications occur when an organism is able to use its own products as food, as is the case with some acetic bacteria. They will at first produce considerable amounts of acetic acid and after a while they oxidize the acid completely. It becomes impossible to account for microbial activity by a chemical equation when several organic compounds are decomposed at the same time as is found to occur in some foods, as butter, cheese, ensilage and in sewage. It is also impossible to formulate exactly decompositions which are caused by mixed cultures. The complications become so great and the relations between different organisms are so little known that it is useless to make the attempt.

Physiological Variations

The great variability of microörganisms in morphological respects has already been pointed out in Part I of this book. A similar variation and adaptation are noticed in their physiology, especially with the food substances of bacteria and consequently with their metabolic products. Microörganisms change their physiological properties very readily with the environment; the new variety may keep its acquired properties for some time even if brought back to the original conditions. It is stated frequently that microörganisms tend more toward variations than the more complex organisms. It should be considered, however, that the experiences in the variations of green plants and animals are based on individuals, while in the case of microörganisms these experiences are gained almost always from millions of cells. A simple illustration is the

development of bacteria in salt solutions. If a broth culture of *B. coli* is transferred into broth containing 8 per cent of salt, a large number of cells will die, often more than 99 per cent. The surviving bacteria begin to multiply after a certain length of time and a new variety is created which can tolerate the salt. At first, only about one out of one hundred cells had the power to tolerate salt, but, since the dying cells are not usually counted or considered at all, it is customary to say that bacteria easily adapt themselves to an 8 per cent salt solution. If only one single plant out of one hundred could be adapted to a certain high temperature, it could not be said that it adapts itself easily. This mistake is quite commonly made with microörganisms.

The best illustration for the variability of cultivated microörganisms is the enormous number of varieties of *Saccharomyces cerevisiæ*. Nearly every large brewery has a yeast type of its own which differs from others by the amount of alcohol and aromatic substances produced, by time and optimum temperature of spore-production, by the appearance of the budding yeast in the hanging drop, and also in other respects. The cultivated organisms are not alone in showing this tendency toward variation. The transferring of a soil or water bacterium into the ordinary laboratory media is a complete change of conditions; the different cells of the same species may react differently and give several varieties. A lactic bacterium on meat medium without sugar does not thrive well in the first generations, but it gradually becomes able to grow on this medium. By this treatment, it loses gradually the power of producing acid and does not thrive as well in milk. The attenuation of pathogenic bacteria by cultivation on media, as potato, very different from the blood and muscle upon which they grow most naturally, or by growing them at low temperature, or above the maximum, furnishes another example. The decrease and finally the entire loss of pathogenicity is caused by a change of metabolism, by a loss of the power to produce toxin.

As by certain diet the metabolism can be changed, so certain physiological properties of bacteria can, by proper cultivation, be increased. By the frequent transferring of an organism on gelatin, its liquefying qualities can be increased, provided it had some at the start. By continued passing of a bacterium through an animal, its virulence can be increased. Strains of bacteria which will produce a very high acidity can be bred; this is illustrated by the quick-vinegar process

and by the strong alcohol-producing yeasts of the distillery process. By continued cultivation of an organism upon a certain medium, it will become so acclimatized that it degenerates readily when the conditions become unfavorable. Such specifically trained strains of microörganisms are used in alcoholic and lactic fermentation, in pathogenic bacteriology and in the inoculation of leguminous plants with nitrogen-fixing bacteria.

Products from Nitrogen-free Compounds

Sugars.—It would be entirely beyond the limits of this book to give an account of all the different ways in which sugars and other compounds can be decomposed by microörganisms. It is much more important, for the beginning bacteriologist, to acquaint himself with the main types of sugar fermentations, and with the characteristics of the organisms which bring about these changes.

In the action of microörganisms many distinguish somewhat crudely six common types:

Complete oxidation.
Partial oxidation.
Alcoholic fermentation.
Lactic fermentation.
Acid gas fermentation.
Butyric fermentation.

Most of these types have been mentioned previously.

Complete oxidation of carbohydrates is observed most commonly among molds and mycodermas, and also in a few bacteria, *e.g.*, in *Azotobacter*. It is possible only where there is a ready oxygen supply, as, *e.g.*, in soils of an open texture, in trickling filters, and on the surface of decaying fruits.

The *incomplete oxidation* is, as a rule, more common in nature. Frequently microörganisms produce first an incomplete oxidation, but later oxidize the intermediate products completely. The molds are typical examples. *Aspergillus niger* is noted for its formation of oxalic acid. If it is grown in a sugar solution, it will bring about at first a rapid increase in acidity, but after a while, it decreases again, when the acid is oxidizing completely. The following processes may be noted:

$$C_6H_{12}O_6 + 9O = \underset{\text{Oxalic acid}}{3(CO_2H)_2} + 3H_2O$$

$$(CO_2H)_2 + O = 2\,CO_2 + H_2O$$

The intermediate product can be accumulated by precipitating it with lime which neutralizes the acidity. This principle is used in the commercial manufacture of citric acid by *Citromyces*, a mold closely related to the genus *Penicillium*. This mold oxidizes sugar to citric acid according to the following equation:

$$C_6H_{12}O_6 + 3O = \underset{\text{Citric acid}}{C_6H_8O_7} + 2H_2O$$

This fermentation is much more complicated than this equation indicates, on account of the entirely different chemical structures of citric acid and dextrose. The practical yield in the factory is only about one-half of the theoretical, since complete oxidation cannot be avoided altogether.

The oxidation processes, just recited, can take place only in the presence of oxygen; the other four types of carbohydrate decomposition require no oxygen, and take place as well in the absence of oxygen; the butyric fermentation is brought about only in the absence of oxygen.

Alcoholic fermentation is caused only by yeasts and a few molds; no bacterium produces alcohol according to the well-known equation mentioned above. Alcohol is formed by several bacteria but only in small quantities and always together with several acids; this is a distinctly different type of decomposition.

In the above groups and the following groups of microörganisms, there appears to be a close agreement between the morphological characters of the organisms involved and the specific type of fermentation. Practically all the alcoholic organisms are yeasts, and the lactic acid-producing organisms are streptococci or closely related bacteria.

The lactic bacteria as they are briefly named, such as are responsible for *lactic fermentation*, are readily recognized by their scanty growth on agar, and their excellent growth in milk, bringing about a solid curdling in one to three days. They change sugar to lactic acid only.

$$C_6H_{12}O_6 = 2C_3H_6O_3$$

No gas and no volatile acids are formed by these bacteria. The best-known representative of this group is the organism which causes the normal souring of milk. It was originally called *Bacterium lactis acidi*, but on account of its very close relation to the streptococci, it is more commonly now named *Streptococcus lacticus*. Many streptococci will produce the true lactic fermentation.

The last two groups of organisms, alcoholic and lactic, represent complex fermentations. There are several products formed, and as has already been pointed out in the paragraph on the equation of fermentations, the entire fermentation cannot be described accurately by one equation, for different fermentations operate independently and simultaneously in the same cell. Under slightly different experimental conditions the one or other of these simultaneous fermentations may be favored, accordingly a varying proportion of the products are formed.

The typical representative of the acid-gas forming group of microorganisms which cause *acid-gas fermentation* are *B. coli*, and its near relative, *Bact. aerogenes*. Many of the gas-formers in nature belong in this group; the bacteria of the fermentations of pickles, sauerkraut, salt-rising bread, the gassy fermentation of milk are some of the many representatives. They are distinct rods, with good surface growth, and do not liquefy gelatin. They are commonly spoken of as the coli-aerogenes group. Some of them have peritrichiate flagella, while others are not motile.

The fermentation of dextrose brought about by these organisms has been described originally by Harden in the equation:

$$\underset{\text{Dextrose}}{2C_6H_{12}O_6} + H_2O = \underset{\text{Lactic acid}}{2C_3H_6O_3} + \underset{\text{Acetic acid}}{CH_3CO_2H} + \underset{\text{Alcohol}}{C_2H_5OH} + 2CO_2 + 2H_2$$

Harden himself stated later that this equation holds only for one strain, and that we have several different strains distinguished by a proportion of products quite different from the one suggested by the equation. Recently Kamm has shown that a good mineral food (probably phosphates are the essential agent) favors a formation of gas and of volatile acids, while a scant supply of minerals causes the bacteria to produce mainly lactic acid. We must assume, therefore, at least two simultaneous independent fermentations:

$$C_6H_{12}O_6 = 2C_3H_6O_3$$

and

$$C_6H_{12}O_6 + H_2O = CH_3CO_2H + CH_3CH_2OH + 2CO_2 + 2H_2$$

The first equation is already known to us; it is the true lactic fermentation. The second equation may be divided still further into several simpler equations.

B. typhosus, causing typhoid fever, is closely related to *B. coli*, but

does not form gas. It forms, however, formic acid, HCO_2H, which, if decomposed, would give $H_2 + CO_2$.

The last type of sugar fermentations is the *butyric fermentation*, in which butyric acid is the most conspicuous, but not the only fermentation product. Acetic acid, hydrogen and carbon dioxide, and, with some organisms at least, ethyl and butyl alcohols are formed along with butyric acid. As already mentioned in the paragraph on the equation of fermentation, Kruse believes this fermentation to consist of several simultaneous fermentations, of which the most interesting at this stage is the one showing the formation of butyric acid.

$$C_6H_{12}O_6 = 2H_2 + 2CO_2 + C_4H_8O_2$$

The organisms producing butyric acid are mostly strictly anaerobic spore formers with a tendency to form spindle-shaped cells; they stain bluish-black with iodine and Bredemann gave the clostridium group one species name, *B. amylobacter*, as he found no distinct and characteristic differences between the many strains which he studied. Many members of this group have the ability to fix nitrogen, *i.e.* to build up their protoplasm without using any sources of nitrogen other than nitrogen gas. Most of the so-called "*Clostridium*" species belong in this group. Butyric acid is also formed by *B. tetani* and by *B. botulinus*, the latter of which causes the most dangerous kind of meat poisoning.

Of other sugar fermentations may be mentioned here only by name, the slimy fermentations, as manifested in ropy milk and the mannit fermentation. The latter is one of the very few reduction processes brought about by bacteria, and one which causes trouble in wine.

What has been stated broadly for sugars holds to some extent true also for the alcohols derived from sugars, including glycerin. Many bacteria fermenting dextrose can also ferment mannit and glycerin with a slight variation of the products, but some do not do this.

Among disaccharides there is a great variation of fermentation. Some groups ferment lactose readily as the coli organisms and *Strept. lacticus*, while among yeasts, fermentation of lactose is rare. Practically all yeasts ferment saccharose, however, and among the lactic bacteria and the coli group many strains cannot ferment saccharose.

STARCH.—Quite different is the fermentation of the insoluble carbohydrates of which we can mention only starch and cellulose. Insoluble

compounds can be fermented only after being made soluble by an enzyme, the amylase (see mechanism of metabolism). Amylase is produced by most molds, by none of the fermenting yeasts, by a few torulas, and perhaps mycodermas, and by a great many of the bacteria. The sugar thus produced from starch is decomposed according to the main types mentioned under sugars. The lactic bacteria and the coli bacteria do not attack starch, but some acid-gas fermentations of starchy foods do take place. Butyric fermentation of starch is common. Alcoholic fermentation can be accomplished only by some of the *Mucors*, and *Aspergilli*.

CELLULOSE is decomposed only by very few organisms; these must be very active and very numerous, to judge from the enormous amounts of cellulose produced and destroyed every year on earth. Molds and higher fungi play probably the main rôle in its decomposition; the products have not been determined, but we may well assume a complete oxidation, since no intermediate products have ever been mentioned. No yeast is known to decompose cellulose, and among the bacteria we find but very few species. Some species have recently been isolated which decompose cellulose in the presence of air; the products have not been determined; we can, however, assume a partial oxidation, eventually a complete oxidation. Besides the aerobic fermentation, we have two types of anaerobic fermentation which are ordinarily described as the hydrogen fermentation and the methane fermentation. In these fermentations the gases mentioned, together with carbon dioxide, are liberated, and butyric and acetic acids are formed at the same time. The marsh gas of the marshes originates in this way.

Summing up all the products formed from carbohydrates, we find several acids, among them lactic and acetic acids most commonly, and ethyl alcohol, rarely other alcohols, besides carbon dioxide, hydrogen and water. The variety is not so great, but with these few compounds, a number of different combinations are possible, and the complication of the study of such fermentations lies mostly in the simultaneous formation of several of the compounds.

ACIDS AND ALCOHOLS.—The organic acids and alcohols can be decomposed further by bacteria and molds, also by some yeasts, to simpler compounds. Ordinarily, this decomposition consists in the complete oxidation. Thus, *Oidium lactis* will destroy the lactic acid of sour milk and of soft cheeses by complete combustion.

$$C_3H_6O_3 + 6O = 3CO_2 + 3H_2O$$

By the same process, the acidity of sauerkraut, ensilage, pickles is reduced by mycoderma species. Another *Mycoderma* is known to destroy acetic acid and thus spoil vinegar or fruits and vegetables kept in vinegar; the yeast grows in a thin, dry white scum over the surface, and oxidizes the acetic acid.

$$CH_3CO_2H + 4O = 2CO_2 + 2H_2O$$

The oxidation of alcohols is not always complete. Especially ethyl alcohol is usually oxidized first to acetic acid; this is the common vinegar fermentation. Many different kinds of vinegar bacteria are known, some forming gelatinous masses of cell membranes called mother-of-vinegar, while others remain as separate small cells. They all oxidize alcohol first to acetic acid.

$$CH_3CH_2OH + 2O = CH_3CO_2H + H_2O$$

But most of them will oxidize later the acetic acid completely to carbon dioxide, after the alcohol is all exhausted, unless the oxygen supply is shut off. This behavior reminds one of the formation and destruction of oxalic acid by *Aspergillus*, mentioned previously. It may be remarked here that the vinegar bacteria cannot attack the sugar directly to any appreciable degree, and the manufacture of vinegar from sugar requires two agents, the alcohol-forming yeast, and the alcohol-oxidizing bacterium.

Some of the acids can also undergo an anaerobic fermentation. This is possible only with hydroxy-acids. The fermentation of the calcium salt of tartaric acid has been the first anaerobic fermentation observed by Pasteur, and the fermentation of lactic acid to butyric acid has a reputation for its chemical peculiarity. A compound with four carbon atoms is formed from a compound with only three carbons, a very unusual thing in fermentation.

Fats.—The decomposition of fats is comparatively simple. All fats are glycerides of organic acids, and if they are attacked at all by micro-örganisms, they are first split into glycerin and free acid.

$$\begin{array}{lllll} H_2C-O-CO-C_{15}H_{31} & HOH & H_2COH & HO_2C\cdot C_{15}H_{31} \\ | & & | & \\ HC-O-CO-C_{15}H_{31} + HOH = & & HCOH + & HO_2C\cdot C_{15}H_{31} \\ | & & | & \\ H_2C-O-CO-C_{15}H_{31} & HOH & H_2COH & HO_2C\cdot C_{15}H_{31} \\ \text{Fat} & \text{Water} & \text{Glycerin} & \text{Acid} \end{array}$$

This brings about the liberation of three molecules of free acid from a neutral fat molecule. It is customary to test for the splitting of fat by determining its acidity. The glycerin is readily used up by the microörganisms, while the fatty acids are oxidized but very slowly.

The number of organisms which can attack fat is quite small. Most molds can destroy it; one torula has been found in butter which attacks it, and perhaps a dozen species of bacteria will do the same, among them *B. fluorescens* and *B. prodigiosus*, which cause occasionally the rancidity of butter.

Products from Nitrogenous Compounds

On account of the complexity of the protein molecule, the products of protein decomposition by microörganisms are little known. Some products are conspicuous through their odor, others can be told by certain color reactions, but as we cannot, at the present, give the structural formula of proteins, there is no possibility of stating protein decompositions in equations similar to those of carbohydrate fermentations. The discussion must be limited, for this reason, to the enumeration of the most important products, and to the general types of decomposition.

As in the carbohydrates, soluble compounds are more easily decomposed than the insoluble. The keratin bodies of hair, epidermis and horn are slowly attacked by a very few organisms. Gelatin, casein and serum albumin are more readily decomposed, though their solubility is quite limited. Peptones which are readily soluble are used by the vast majority of microörganisms. Of interest in this connection is the fact that the fresh white of egg is poisonous to most bacteria, and fresh blood and animal tissues as well as freshly drawn milk have also germicidal properties which are lost by heating or upon standing.

Protein bodies are as numerous as plants and animals. Each species of organism seems to have its particular protein which differs from that of other species. With the more highly developed organisms, there are several distinctly different proteins found in the same individual in different parts of the body. The constituents, carbon, oxygen, hydrogen, nitrogen, and sometimes sulphur and phosphorus, can be determined in their relative amounts without, however, furnishing any knowledge of the structure of the molecule. The molecular weight of

proteins is estimated to be at least 10,000, while the weight of the very large molecule of saccharose is only 342. The protein molecule can be broken up into smaller molecules. This cleavage is generally believed to be a hydrolytic process similar to the decomposition of starch to maltose. The first products of protein decomposition do not differ essentially from the original protein, but they can be hydrolyzed again and again, until finally products of a crystalline nature are found which are well-defined chemical bodies. Among the very first products of protein degradation it is usually impossible to determine single compounds, but several groups of compounds may be separated by certain precipitants, as acetic acid, ammonium sulphate, zinc sulphate, copper sulphate, tannic acid and others. In order to determine the degree of protein degradation, *e.g.*, in the analysis of cheese, it is customary to determine the nitrogen of compounds precipitated by these various reagents, and state it in percentage of the total nitrogen. Thus the terms "water-soluble nitrogen," "acid-soluble nitrogen" and others originated, meaning the nitrogen of the compounds soluble in water or in acid respectively. Some of these groups of degradation products have been named and defined more accurately, of which the albumoses and peptones are the most common and best described compounds. Their chemical nature and structure is, however, just as little known as that of the protein bodies. We speak of peptonisation of proteins, *e.g.*, in the clearing of milk or the gelatin liquefaction, meaning that the insoluble protein has been made soluble.

The amino-acids are the first well known compounds of protein decomposition. They are organic acids, in which a hydrogen atom is substituted by a NH_2 radical. Some of them are simple compounds, as the amino-acetic acid NH_2CH_2COOH and also the amino-capronic acid usually called leucin $(CH_3)_2CH\ CH_2\ CH(NH_2)\ COOH$. Others are of a more complex nature, such as the tyrosin or hydroxy-phenyl-aminopropionic acid, $C_6H_4(OH)\ CH_2CH\ (NH_2)\ COOH$, and the tryptophan or indol-amino-propionic acid, $C_8H_6N\ CH_2CH(NH_2)\ COOH$.

Of other nitrogenous products which are not amino-acids, a few are of striking significance. The very disagreeable odor of putrefying proteins and of excreta is due to indol (C_8H_7N) and methyl-indol or skatol ($C_8H_6N{\cdot}CH_3$). Indol gives a rose color with nitrites in acid solution, and this convenient reagent is used in the identification of

bacteria. Another group are the amins. The simplest amins are the methyl-amins, of which the tri-methylamin $(CH_3)_3N$ is produced by several bacteria. The fishy odor of the brine of salted herring is largely due to this compound. In this group belong also a large number of the so-called *ptomains*.

The ptomains (page 491) are alkaloid-like bodies of basic character and of more or less well-known structure. Some of them are notorious for being very strong poisons, while others are quite harmless. These bodies are called ptomains because they were first discovered in putrefying corpses. The best-known compounds of this character are the putrescin or tetra-methylen diamin $[NH_2(CH_2)_4NH_2]$ and the cadaverin or penta-methylen-diamin $[NH_2(CH_2)_5NH_2]$, which can scarcely be considered poisonous. The methyl-guanidin

$$HN = C\begin{matrix} NH_2 \\ \diagdown\diagup \\ NHCH_3 \end{matrix}$$

may be mentioned as an example of a very poisonous ptomain. Another poisonous ptomain is the neurin $CH_2 = CH - N(CH_3)_3OH$ which has been found frequently as a product of putrefaction.

Ammonia is the end product of protein decomposition, as far as the nitrogen-containing fragments of the protein molecule are concerned. That ammonia is formed by many bacteria, is well known. In some decaying proteins, *e.g.*, in old Camenbert cheese, ammonia can be very easily detected by the smell. As all proteins contain many amino-groups as well as acid-amid groups, it is easily understood how the ammonia originates through the hydrolysis of protein. In the complete oxidation of proteins, the nitrogen is always left as NH_3 or $(NH_4)_2CO_3$ respectively, never, so far as known, in any other form. No bacterium is known to produce urea, as most of the higher animals do.

In the products of protein degradation mentioned above only those compounds have been considered which contain nitrogen. It is quite evident, however, that in the cleavage of the large and complex protein molecules, certain parts of the molecule will contain no nitrogen. Many organic acids, like acetic, butyric, capronic, benzoic and phenyl-acetic acids are quite generally found among the products of putre-

faction. Alcohols too, especially benzene derivatives like phenol and cresol, are not unusual. Gas is often formed in putrefaction, especially carbon dioxide and hydrogen; occasionally these gases are mixed with traces of nitrogen and methane.

Many protein compounds contain, besides the organic elements, larger or smaller amounts of phosphorus and sulphur. The phosphorus compounds may be changed to phosphine (PH_3), which is a gas of a strong disagreeable garlic odor. Generally, however, the phosphorus of protein after its degradation is found as phosphoric acid (H_3PO_4). Very little is known about the phosphorus of organic compounds and the changes it may undergo in the putrefactive process.

The sulphur of proteins is commonly changed to hydrogen sulphide (H_2S). Some microörganisms are able to form mercaptan (CH_3SH), a compound of very foul penetrating odor.

After this enumeration of the products, the main types may be considered briefly; since much less work has been done on protein decomposition than on carbohydrate decomposition, the groups are not so well defined. We might consider the following types:

Complete Oxidation.—This is brought about by many molds, by yeasts if they depend upon proteins only, and by many bacteria, of which the large, aerobic spore-forming rods, such as *B. mycoides*, are the main representatives. The products of oxidation are CO_2, H_2O, NH_3 and H_2SO_4. The nitrogen is never changed to any oxidation product, but is found as NH_3, while the sulphur is oxidized.

Incomplete oxidation is caused by other bacteria, and perhaps molds and yeasts. Quite a large number of organisms live on sugar-free media if they have oxygen, but they do not oxidize their food completely. We can distinguish at least three different groups of microörganisms here.

B. proteus is the collective name for a number of closely related forms which belong to the most common organisms found on decaying organic matter, especially when protein is abundant. They produce leucin, tyrosin and tryptophane, but no skatol, or phenol. Indol and hydrogen sulphide are formed in certain media. Less important, but also very common are the pigment-forming rods among which *B. fluorescens*, *B. prodigiosus*, *Ps. pyocyanea* are the best-known representatives. Their metabolism is a little different; amins and ammonia are formed, while hydrogen sulphide, phenol and indol are absent.

As a third group, *B. coli* may be mentioned which forms indol, but no ammonia from peptone, and whose proteolytic powers are very weak as it does not even liquefy gelatin.

Anaerobic decomposition of proteins is limited to very few species; there is a great difference in the availability of proteins and of carbohydrates as a source of energy, protein being available only to a few species, most of these preferring carbohydrates if they are present together with protein. *B. putrificus* is the main representative, but other forms exist. *B. putrificus* is strictly anaerobic, and a spore former, very common in nature. Among the products are skatol, hydrogen sulphide, ammonia and other very offensive compounds.

UREA, URIC ACID, HIPPURIC ACID, are the end products of protein metabolism of the higher animals. The decomposition of urea to ammonium carbonate has been mentioned in several places, mainly on page 146. It is a simple hydrolysis

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3.$$

This change can be brought about by only a few bacteria which are commonly grouped together as "urea bacteria." These organisms have hardly anything else in common, however, and the group is not a well-defined one. There are rods and coccus forms, motile and non-motile organisms, spore-formers and non-spore formers, and even molds have recently been found to hydrolyze urea. All urea bacteria can live without urea, feeding on organic matter like other bacteria, but most of them require an alkaline medium.

Hippuric acid is split by certain bacteria to benzoic acid and amino-acetic acid which can be oxidized completely. Uric acid can be changed in several ways. In some of these changes, urea is found as an intermediary product.

PRODUCTS FROM MINERAL COMPOUNDS

Minerals are used by microörganisms for cell construction almost exclusively; consequently, they do not leave the living cell-like fermentation products. But a few organisms can actually decompose mineral matter and when this takes place mineral products are secreted. Two main processes can be distinguished, oxidation and reduction.

OXIDATIONS are the result of the organisms seeking a supply of energy. Several oxidations of minerals have been indicated previously, as the oxidation of ammonia to nitrites, of nitrites to nitrates, of hypo-

sulphites to sulphates, of hydrogen sulphide to sulphur and of sulphur to sulphuric acid, of ferrous salts to ferric salts. All these microbial changes are simple processes and can be followed by chemical analysis much more easily than organic fermentations. The organisms which cause these changes, do not, as a rule, thrive in organic substances and for this reason pure cultures can be obtained only with difficulty. Their activity is of great importance in soil fertility.

Reductions of minerals, too, are of great significance. As a typical example, nitrates may be reduced to nitrites, to ammonia, to nitrogen gas, and, rarely, to nitrogen oxides. The reduction may be performed either by the direct removal of oxygen, or by the formation of free oxygen. The reduction of nitrates to nitrites can be written in the following three ways:

$$KNO_3 - O = KNO_2$$
$$KNO_3 = KNO_2 + O$$
$$KNO_3 + 2H = KNO_2 + H_2O.$$

The result in all three cases is the same. Many bacteria can reduce nitrates to nitrites or to ammonia. A few can reduce them to nitrogen. These "true denitrifiers" are found in soil and in old manure. Their reducing process is as follows:

$$Ca(NO_3)_2 - 5O = CaO + 2N.$$

Nitrates are reduced through the efforts of the organism to secure a supply of oxygen. The denitrifying bacteria have strong oxidizing properties; they take oxygen from all sources possible. If cultures of denitrifying bacteria are well aerated, as in soils with a proper moisture content, they scarcely attack the nitrates, while they will reduce them in ordinary liquid cultures so fast that the escaping nitrogen gas forms a froth on top of the nitrate solution. Denitrifying bacteria need the oxygen to oxidize organic matter. They cannot live without organic food.

Sulphates are reduced in a very similar way to hydrogen sulphide

$$H_2SO_4 - 4O = H_2S.$$

Tap-water, containing calcium sulphates, often forms hydrogen sulphide if shut off from the air for some time.

While only a few bacteria reduce sulphates, many reduce sulphites or sulphur to hydrogen sulphide. The potassium and sodium salts of selenic and telluric acid (H_2SeO_4 and H_2TeO_4) are reduced by certain organisms and not by others. The reduction results in a colored precipitate; this reaction has been suggested as a diagnostic means to distinguish different species. The reduction of arsenious oxide to arsin (AsH_3) is used as a very delicate test for arsenic; it is applied in the detection of arsenical poisoning. The material to be tested is sterilized and inoculated with *Penicillium brevicaule* (page 52, the "arsenic mold"). This will reduce most arsenious compounds to arsin ($As H_3$) or to diethyl arsin, $As H(C_2H_5)_2$, both of which are easily recognized by their very pronounced garlic odor.

Unknown Products of Physiological Significance

Among the products of microbial action, there are certain substances which must be mentioned because of their importance, though their quantity is insignificant compared with the ordinary products of fermentation. These substances can be divided into four groups: *pigments*, *aromatic compounds*, *enzymes*, and *toxins*. The chemical structure of pigments and of many aromatic substances is scarcely known; and as far as enzymes and toxins are concerned, it is not even determined whether or not they are of protein nature. The last two groups are known only by their actions, while the pigments are very conspicuous and cannot possibly be overlooked.

Pigments have naturally attracted the attention of microbiologists ever since pure cultures were known, and many investigators have tried to explain the nature and the meaning of pigments. All experiments concerning the purpose of pigment-formation by microörganisms have been without results. It is not known that the pigment is of any material advantage to bacteria; for it is possible to cultivate colorless strains of pigment bacteria which grow apparently as well as the original pigmented culture. Again, pigments cannot take the place of the chlorophyl in plants except perhaps the bacteriopurpurin of the purple bacteria. It does not even protect the cells against intense light, because the pigmented organisms are not more resistant than the corresponding colorless "sports." The only exception are the colored spores of the molds, especially *Penicillium* and *Aspergillus*, which are very resistant to light, while the spores of *Oidium* are killed just as easily as

the mycelium. Pigments cannot be considered as reserve substances, since many pigments are excreted and remain outside the colorless cells. Pigment production may be incidental. It is possible that the waste products of certain organisms happen to be colored.

After Beyerinck, the chromogenic bacteria may be divided into three classes:

1. *Chromophorous bacteria*, in which the pigment is placed in the cell and has a certain biological significance analogous to the chlorophyl of higher plants. In this division belong the green bacteria discovered by Van Tieghem and Engelmann and the red sulphur bacteria or purple bacteria.

2. *Chromoparous or true pigment-forming bacteria*, which set free the pigment as a useless excretion, either as a color-body or as a leuco-body which becomes colored through the action of atmospheric oxygen. The individuals themselves are colorless and may under certain conditions cease to form pigments. To this class belong *B. prodigiosus*, *B. cyanogenes*, *Ps. pyocyanea*, and others.

3. *Parachrome bacteria*, which form the pigment as an excretory product but retain it within their bodies, as *B. janthinus* and *B. violaceus*.

When the pigment is soluble in water, as those produced by *Ps. pyocyanea* and the fluorescent bacteria, it diffuses through the medium. When the pigment is not soluble, it either lies within the cell wall or between the individuals.

This classification furnishes some details concerning the methods of pigment production, which depends upon the presence of certain media. According to Sullivan, sometimes certain mineral salts, sometimes sugar will stimulate chromogenesis. The same is true with molds. Very brilliant colors appear with certain species of molds if grown on cellulose or on fat, while on gelatin the pigment is not produced. The temperature is an important factor. A large number of chromogens produce no pigment when grown in the incubator. It is possible to obtain non-pigmentation with many species by propagating them through many generations at high temperatures. Oxygen also is necessary for the chromogenesis of many bacteria. Some need a short exposure to daylight in order to produce their pigment, while cultures grown in absolute darkness may remain colorless. Strong sunlight however, will check pigment production in the same degree as do antiseptics and other harmful influences.

The chemical nature of microbial pigments is little known. They æ distinguished according to the solubility in various liquids, water, æohol, ether, chloroform, benzol, and other solvents, and according t the change of color caused by acid and alkali. A group of *rotin bodies*, named because of their similarity to the pigment c carrots, the *prodigiosin bodies*, named after *B. prodigiosus*, the

G. 101.—Bacteriopurpin, from a *Rhodospirillum*, crystallized from a chloroform solution. (*After Molisch.*)

iorescent pigments and perhaps a few other groups are distinguished, it their chemical nature is rather vague as yet. The absorption of stinct lines of the spectrum by solutions of these pigments is claimed be a very reliable means of distinguishing the pigments of different ecies.

Aromatic substances constitute another group of metabolic prodts. The chemical analysis accomplishes more with these compunds than with pigments, since they are frequently well-known mpounds. The main difficulty arising in their identification is in e very minute quantities of the products available. Some substances ith strong, mostly very disagreeable odors have already been menoned: indol, skatol, hydrogen sulphide, mercaptan, the amins and mmonia, butyric acid, and some of the higher alcohols. There reain to be mentioned certain oils and esters giving rise largely to easant aromas. The formation of aromatic oils has been established though their nature is entirely unknown. The same is true with the ters. The substance causing the fishy flavor in butter is volatile ith steam and is neither of an alkaline nor acid nature. The strong lor of freshly plowed earth is caused by an *Actinomyces;* the odor

can be traced to a very volatile oil the nature of which has not been determined. The aroma of fermented liquids—wines, beers, and many others—is partly due to compounds constituting the fermenting material, and partly to the fermenting agent. Some yeasts are known to produce fruit-esters, as succinic-acid-ethylester and the corresponding esters of malic and other acids. Besides, some glucosides may be split and traces of hydrocyanic acid and benzoic acid may be liberated. The change of flavor with the aging of wines is probably more a chemical than a biochemical change.

Enzymes and Toxins.—Among the most interesting and least understood products of microbial action are the *enzymes* and the *toxins*. These two groups are related in many respects. The enzymes will be discussed extensively in the following chapter and toxins are treated more extensively on pages 575, 676. Toxins and enzymes are formed by the cells in such small quantities that they would never have been discovered by ordinary chemical means were it not for the unusual effects which they produce, the enzymes acting upon food substances and the toxins acting physiologically upon organisms. Toxins and enzymes are chemically unknown. It is assumed that they are chemical bodies, but even this has not been proved. A pure toxin has never been obtained and we have no criterion for its purity. The presence of a toxin is recognized only by an animal test and in this way the comparative concentration can be determined approximately. Such standardization of toxin solutions is only comparative, however, and gives no clue as to the actual amount of toxin present. Not all animals are sensitive to all toxins. It is quite possible that all bacteria produce compounds with chemical qualities similar to toxins, and only a few of them happen to react upon men or animals.

Toxins are not always the product of microbial action. Vegetable toxins or *phytotoxins* are known, among which the ricin of the castor oil bean is perhaps the most studied representative. The best-known zoötoxin is the rattlesnake poison. These non-microbial compounds have the same quality as the microbial toxins—they are extremely poisonous. Toxins are the cause of disease in diphtheria, tetanus and botulism. If a culture of these organisms is filtered through a porcelain filter which removes all bacterial cells, the filtrate injected into an animal will cause the disease with all its accompanying symptoms though there are no microörganisms introduced into the animal body

the filtrate is heated, however, no effect will take place after the injection, because heat destroys the toxin. The amount of toxin that will kill an animal is extremely small. .000005 mg. of the purest tetanus toxin will kill a mouse, .0007 mg. of ricin will kill a rabbit, less than 3 mg. of tetanus toxin will kill an adult man. The body of an animal or man forms an anti-body against the toxin which neutralizes its poisonous action. Anti-bodies are also formed against enzymes injected into an animal.

Toxins are very sensitive to heat. A short exposure to temperatures between 80° and 100° will inactivate them. They are also very sensitive to light. While some toxins are secreted, others are retained within the cells of microörganisms, and never leave them until the cells die or disintegrate. Ptomains, which are also metabolic products of microörganisms and sometimes cause poisoning, differ from the toxins in their resistance to heat and light (page 171). Ptomains differ in no way essentially from ordinary organic compounds; the animal or human body produces no anti-ptomains to counteract their poisonous effects. There is no chemical relation whatever between toxins and ptomains, and the physiological effects are also quite different, though they both cause poisoning.

Toxins are not essential products of the metabolism of pathogens. Strains of pathogenic bacteria can be bred which do not produce toxins as chromogens can be bred without pigment, or lactic bacteria which do not produce acid. The strains which lose their pathogenicity grow better on artificial media but are less able to produce disease in the animal. They may regain the power of producing toxin if passed through the body of the animal. The real object of toxin production by microörganisms is not known; the microörganisms derive no apparent benefit.

Factors Influencing the Type of Decomposition

In the chapter on products of metabolism, it has been shown that the same compound can be decomposed in many different ways, and the question may well be asked what decides the type of decomposition. Since bacteria are widely distributed, it must be expected that there are certain conditions which are most favorable to a given type of fermentation, while under changed conditions, other types are more

likely to dominate. The fact that sugar in cider nearly always undergoes alcoholic fermentation, while in milk it undergoes lactic fermentation, has its reason in the physiology of the bacteria, and in their reaction upon the environment.

Cider is acid, and acid is not well suited for the growth of most bacteria. The vinegar bacteria can grow in fruit juices, and a few other bacteria, especially those causing trouble in wine, are not retarded by fruit acids, but the common types attacking proteins and causing organic decay are not able to grow on fruits. Yeasts, however, and molds thrive well only in acid media. They can exist in neutral solutions if in pure culture, but in nature they are easily crowded out by bacteria. Acidity of the medium is therefore one of the most important factors regulating the type of microbial decomposition. This principle is commonly utilized by preserving foods of all kinds in vinegar, and by making butter from sour cream rather than sweet cream; the keeping qualities of hard cheeses depend upon their acid content.

In acid environment, the two most common types of decomposition are oxidation, complete or incomplete, and alcoholic fermentation. The oxidation is brought about by molds or organisms closely allied to yeasts. The latter are very common on all sour foods, especially on foods containing lactic acid, such as cottage cheese or sauerkraut. The kind of acid decides the type of mold; wherever there is lactic acid there is *Oidium*, while malic and tartaric acids favor *Penicillium* and *Aspergillus*.

If the decaying materials contain no acid, the type of decomposition depends mainly on the presence or absence of carbohydrates, especially sugar. It is an old experience, recently verified through a large number of experiments by Kendall and Walker, that practically all bacteria will decompose sugar in preference to proteins. If a leaf contains sugar and protein (cabbage) the sugar decomposition will be conspicuous, and the protein is not attached very readily. Putrefaction in the presence of sugar or of acid does not take place. Meat will not putrefy if mixed with sugar, while milk putrefies readily if the sugar is removed by dialysis. The three types of sugar decomposition which come into consideration in neutral media, are the lactic, the acid-gas and the butyric fermentations. The latter is a strictly anaerobic fermentation, and thus limited to special conditions. Of the other two, the acid

as fermentation is the most common, and the souring of vegetables f all kinds is due to this type of fermentation (pickles, sauerkraut, nsilage, salt-rising bread). Sometimes the acid-gas fermentation s followed by a butyric fermentation. The true lactic fermentation s not common, and is limited almost entirely to milk. This is ex- lained by the circumstance that the organisms causing this decom- osition are parasitic in their habits, causing disease or living in the ntestine of animals. In the absence of acid and sugars, putrefaction s the most common type of decomposition.

Many factors aside from the chemical composition of the medium re essential. Oxygen has already been mentioned as preventing buty- ic fermentation. It will also prevent the acid-gas fermentation if too bundant. Ensilage is trampled and pressed down to avoid air spaces s much as possible, for molds will outgrow the acid-forming bacteria f air has free access. Absence of oxygen will prevent mold growth, nd for this reason, jelly is paraffined, and butter wrapped tightly into mpermeable paper. The influence of oxygen upon the type of protein nd of cellulose decomposition has been pointed out previously.

The moisture content is of great importance. As will be shown ater, not all organisms have an equal need of moisture; some molds vill grow on foods too dry for bacteria and yeasts. Molds are es- pecially adapted for growing on dry media, as only part of their cell ubstance is immersed in the medium. Their thread formation enables hem to search a dry medium, such as flour, for moisture, the extreme of adaptation being *Rhizopus*, and the construction of the fruiting odies shows that they are destined by nature to be spread by air and wind. It is no wonder that damp organic matter, if it can be de- composed at all, will show molds, and nothing else, regardless of the chemical composition, for there is no competition. Flour, moist seeds, ncompletely dried fruit, damp milk powder will always become moldy. The same holds true with very concentrated sugar solutions such as syrups, jellies and jams, while in concentrated salt solutions, molds cannot thrive, and the torula yeasts are best adapted to such conditions.

A very important part is also the structure of the material. Micro- organisms act mainly upon organic matter, and since this comes from living organisms, it has usually definite structure, exceptions being milk and blood. The structure of all living organisms is such

as to prevent the intruding of microörganisms. The body of plants and animals is surrounded on the outside by tough and dry layers of epithelial cells, and the cavities of the animal body also have their protective membranes. Microörganisms cannot enter the tissues if these membranes are perfectly sound, and we know that, as a rule, the tissues of healthy plants or animals are free from bacteria. Thus, a healthy apple or potato or egg will not be infected and decomposed by microörganisms if handled carefully, meat will begin to decompose on the outside, and the inner parts may be still good when the outer layer is already in a state of decay.

In the plants, each cell is surrounded by its special cell membranes which are a barrier to infecting organisms. If we prick the skin of a healthy apple with a pin infected with yeast, the infection will not spread though we know that yeast will grow most abundantly in cider; in the apple, however, it has no means of spreading from one cell to the other. Molds possess this means; they can puncture cell walls, and forcing their way from one cell to the other, they will soon bring about the rotting of the entire fruit after it once becomes infected. This protection seems especially necessary in the plant's roots which are greatly exposed to injury from insects and other animals in the soil and surrounded by billions of microörganisms. They are attacked only by fungi which can force their way from cell to cell or by bacteria which can dissolve the membranes by means of enzymes and thus cause a softening of the root tissue. The bacteria causing the various rots of vegetables belong to this type.

There is, then, a great variety of factors deciding the type of decomposition of organic matter in nature, and by knowing the chemical composition as well as the structure and other physical conditions it is possible to foretell which group of organisms is most likely to attack the compounds in question.

Another quite important factor, the temperature, will be discussed in more detail in one of the following chapters.

Rotation of Elements in Nature

All organic matter on earth is undergoing continuous change. Organisms grow and decay. The same carbon and nitrogen atoms which constitute the organic world of to-day constituted it thousands

ears ago. The amount of carbon, nitrogen, hydrogen and of all ther elements of life on earth is limited, and the same atoms will e used for the future generations of life that constitute the present. here must be continuous destruction to enable new construction. onstruction is mainly the task of green plants, enabled by the chloro- hyl to use the energy of sunlight in building up organic substances om minerals, water and carbon dioxide. Destruction is caused ainly by animals and other organisms which have to break down rganic matter in order to exist. These two factors keep the atoms of he organic world in perpetual rotation.

In this circulation of the elements it is necessary that all compounds f organic nature be decomposed finally to a form available for plant ood. If this were not the case, the indestructible compound would ooner or later accumulate in such enormous quantities that the lements constituting this body would be removed entirely from general circulation. Let us suppose, as an illustration, that for some nknown reason, all urea bacteria on earth would die. Urea could be decomposed no more, and the plants, unable to use urea as a source of nitrogen in place of nitrates, would get but little benefit out of stable manure. All urea would pass gradually undecomposed into rivers, lakes, and finally into the ocean where it would accumulate continuously. The enormous quantities of nitrogen taken out of circulation would cause a decreasing growth of plants, and life would soon cease because of lack of nitrogen. For this reason all products of living organisms must be further broken up by some other organisms, and we find that the destructive work is to a large extent the task of microörganisms. Many products of organic life cannot be broken down by organisms other than bacteria, and therefore bacteria are absolutely necessary for the circulation of the elements and for life on earth. Bacteria and green plants are an absolute necessity for the maintenance of life, the one breaking down, the other building up, one dependent upon the products of the other; animals, however, could be excluded from the circle without interfering with a continuation of life on earth.

Carbon Cycle.—Carbon is the main element in organic nature, and the study of its cycle might be begun with its simplest compound, the carbon dioxide of the air. It is absorbed in this condition by the green plants, and is changed by the chlorophyl granules of the leaves to

organic compounds of various types, either to carbohydrates (cellulose, starch, sugars) or to fats, or to protein substances, occasionally to organic acids or other compounds. The plants will either die and decay, or will be eaten by animals. In the first case, the decay will be caused exclusively by microörganisms; if the plants are eaten, they will be digested; part may be used to build up the animal body or stored as reserve substances, largely fat and protein. If the animal dies, a decomposition process will take place, which breaks down the organic compounds to simpler products and finally the carbon will be com-

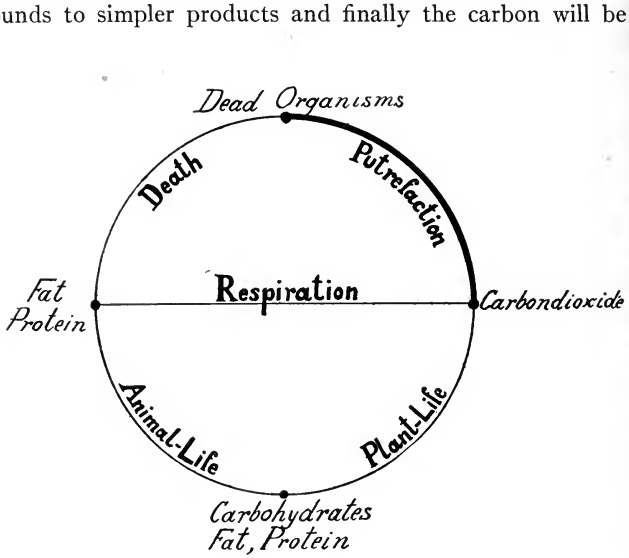


FIG. 102.—Carbon cycle.

pletely oxidized to carbon dioxide. Even the marsh gas which might be liberated in this process will find organisms that oxidize it to carbon dioxide and water. Every product will find an organism to break it up further until it is completely disorganized and the carbon atoms can start the same circulation anew. Undoubtedly as long as organic life has existed on earth, microörganisms have been present, in order to render the dead organic matter again available for plant and animal life. Fig. 102 gives a schematic illustration of the carbon cycle; the microbial activity is marked by heavy lines.

NITROGEN CYCLE.—Nitrogen shows the same continuous change as carbon. Plants take up nitrogen in mineral form usually as nitrates. The plants change this mineral nitrogen to the most complex bodies, proteins, where it is combined with the other elements of organic nature. The plants may be eaten by animals; part of the protein is then digested to urea or hippuric or uric acid, which in turn are readily decomposed by microörganisms to ammonia (Fig. 103). Part of the protein will be stored in the growing animals, and if the animal dies, the body will decay or putrefy, and the nitrogenous compounds of that body will pass through the various stages of decomposition to the final product,

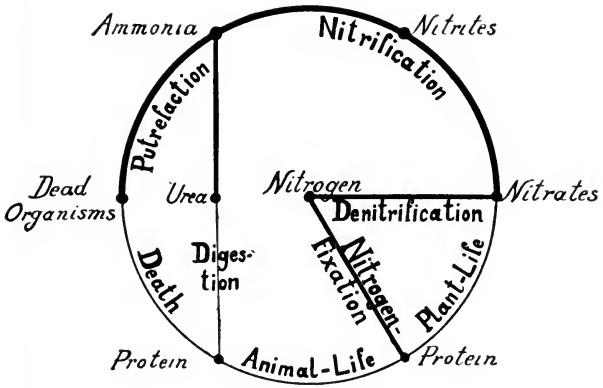


FIG. 103.—Nitrogen cycle.

ammonia. Ammonia is then oxidized to nitrites and nitrates, when the nitrogen cycle is completed.

There is, however, one discrepancy in this cycle. It has been mentioned already that some organisms are able to reduce nitrates to nitrogen gas. This is one of the "leaks" in the rotation of elements which would be disastrous to organic life on earth if there were no means to compensate for the loss of nitrogen in circulation. Imagine what would happen if there were no such compensation. Part of the nitrate in the soil is destroyed, the nitrogen gas escapes into the air and is as indifferent as the nitrogen of the atmosphere lost to organic life forever. More nitrates would be produced from decaying organic matter and would eventually be destroyed. After a certain time, this continuous

loss of nitrogen would become quite noticeable in the growth of plants; there would be a scarcity of nitrogen in soil, since part of it is lost continuously. Finally, the plants would cease to grow because the nitrogen in the soil would be exhausted.

The compensation for this destruction of available nitrogen is found in the nitrogen-fixing bacteria, which, either living in symbiosis with leguminous plants or growing independently in the soil, have the power to use the atmospheric nitrogen for the formation of their own protoplasm. Thus, organic nitrogen is produced from nitrogen gas and the continuance of organic life is guaranteed.

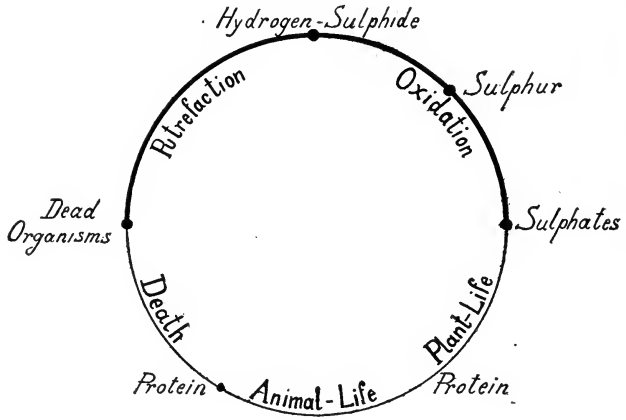


FIG. 104.—Sulphur cycle.

SULPHUR CYCLE.—Little more can be said about sulphur, since the rotation is quite similar to that of nitrogen. Plants will take sulphur usually in the form of sulphates and make protein compounds containing a certain amount of sulphur (Fig. 104). These bodies are either digested by higher animals or broken down by putrefaction to the final product, hydrogen sulphide, which is oxidized by the sulphur bacteria first to sulphur, then later to sulphates.

PHOSPHORUS CYCLE.—The cycle of phosphorus has not been worked out completely, but from the discussion in the last pages, it is plainly seen that a simple cycle very much like the ones above must exist. It

is probably much simpler because phosphorus does not enter as easily into organic compounds as nitrogen.

Physical Products of Metabolism

Production of Heat.—It has long been known that fermentation produces heat. The rise of temperature is usually not very great. In lactic fermentation it amounts to about 1°, in alcoholic fermentation to 2 or 3°, but in certain processes the heat liberated is considerable, as in the fermentation of manure, of ensilage, of vinegar, and in others.

The cause of heat formation is quite evident from the discussion on page 142. Decomposition of organic matter means a liberation of energy which is used for the continuation of life processes; the utilization is, as a rule, incomplete, and a part of the energy appears in the form of heat. The amount of heat produced can be measured directly with the thermometer if great care is taken that no heat is lost by radiation or by evaporation of water.

Much heat is produced in the vinegar fermentation. In the quick-vinegar process (page 546) the temperature rises sometimes as high as 10° to 15° above the temperature of the room and the vinegar manufacturer uses the heat produced by the bacteria to keep the generators at the optimum temperature. If the process is not controlled carefully, the vinegar bacteria are likely to produce sufficient heat to kill themselves.

The heat produced in the fermentation of manure, especially horse manure, is used in the hot-beds to cultivate and force young plants. In the manure pile, great heat production is not desirable because high temperatures will volatilize the ammonia; the tight packing of manure which keeps out the oxygen will prevent too strong bacterial action. The highest temperature in silos which has been recorded is about 70°, but the best silage is secured by keeping the temperature below 50°. Ensilage fermentation is not thoroughly understood, however, and no accurate statements can be made as to the cause of the increase in temperature. Sometimes the temperature in silos does not exceed 35°. The curing of hay is usually accompanied by a rise of temperature. For some time it was believed that the spontaneous combustion of hay was mainly due to microörganisms, but it has been shown recently that even sterile hay will show a rise of temperature under certain

conditions. This does not exclude the formation of heat in hay by microörganisms under other circumstances. The heating of tobacco, of green or moist grain or corn is not of bacterial origin, but due to the respiration of the living plant-tissue.

Production of Light.—The light-producing or photogenic organisms are quite numerous and occur more frequently than is generally believed. The phosphorescence of decaying tree stumps and leaves in the woods and of meat and fish in the cellar are well-known phenomena. The phosphorescence of wood and leaves is generally caused by *Hyphomycetes;* certain mushrooms have this quality in a very high degree. The light of meat and fish is usually generated by bacteria, of which at least twenty-six species have been described.

Many experiments have been carried on in order to discover the nature and origin of the light, but, so far, few results have been obtained. The phosphorescence is due to an oxidation process; all photogenic organisms cease to generate light when the oxygen is removed. As soon as they come into contact with oxygen again, they produce light immediately, and this sudden flashing is used occasionally by physiologists as a very delicate test for oxygen. The light appears to be produced always within the cell; no cell product has ever been found to give rise to light outside the cell. It is possible that a chemical compound is formed in the cell which generates light when in contact with oxygen.

The life processes of the photogenic microörganisms are not necessarily connected with the formation of light. Photogenic bacteria are known to lose the power of light production as the chromogenic bacteria may lose the power of pigment production. Phosphorescence has, like pigmentation also, no bearing upon the development of the cell, and the light-giving compounds may be regarded as incidental waste products. Certain chemical bodies stimulate light production, while others favor the growth only. One of the most important factors in the production of light is sodium chloride.

CHAPTER III

MECHANISM OF METABOLISM

General Theory of Metabolism

Anabolism, Katabolism, Metabolism.—In the introduction to the Physiology of Microörganisms, it was stated that microörganisms need food for at least two different purposes: building material and building energy. They may need it for other purposes also, *e.g.*, for motion. The sum of all changes which the food undergoes in the body, including the deterioration of the cells, is called *metabolism*. Metabolism consists of several separate functions: One of them is the construction of new cells, or parts of cells, called *anabolism*, another the deterioration of cells, called *katabolism*, and the most important quantitatively is the *fermentation* or *respiration*. The fermentation or respiration processes are fairly well understood; many of them can be produced in the chemical laboratory without microörganisms. Katabolism is the sum of many processes some of which are well understood while others are still unknown. The synthetic, anabolic processes of the cell, however, are almost entirely unknown, and we can only speculate regarding the various means by which the cell grows. The explanations of the different cell activities began, as in most other fields of theoretical bacteriology, with a close analogy with animal and plant metabolism, but owing to the comparative simplicity of the microörganisms, they led to the establishment of new facts and theories which proved afterward useful for the understanding of the metabolism of the more complex organisms where the multiplicity of facts prevented a clearer insight into the separate processes.

Intra- and Extra-cellular Fermentation

Decomposition of Insoluble Food.—It has been stated before that many microörganisms feed upon cellulose, starch, fat, gelatin, keratin and other insoluble compounds. It has also been previously stated that microörganisms, with the exception of some protozoa,

depend upon soluble food since they have no means of incorporating insoluble compounds into their protoplasm. The protoplasm, however, must be considered the center of metabolism, and the digestion of food and the formation of energy must take place in the protoplasm if the cell is to profit by it. Since the food cannot diffuse into the cell, and the protoplasm does not diffuse out, the food must be dissolved. This is accomplished by the cell itself by secreting certain agents with peculiar qualities. These agents, the so-called *enzymes*, act upon the insoluble foods, changing them into soluble compounds which then can diffuse into the cell where they are digested or fermented. The final digestion or fermentation of the food must take place within the cell. Energy production outside the cell serves the same purpose as a stove outside the house. The dissolution of insoluble compounds by cell secretions must be considered a preparatory process which has no direct relation to intra-cellular food digestion or fermentation. Enzymes are not produced by microbial cells exclusively. All living cells produce enzymes. They were known before the science of microbiology had been established. In fact, microbial activity was considered for a long time as an enzymic chemical process. Enzymes in the animal and plant body serve largely the purpose of metabolic changes. In the animal body, many enzymes help to dissolve the insoluble food which cannot pass from the alimentary canal into the body except by diffusion through the mucous membrane. There is *diastase* in the saliva which acts upon starch, there is *pepsin* in the stomach and *trypsin* in the intestine, both dissolving protein bodies; there is *ereptase* for the peptones, *lipase* for the fat, *invertase* for the saccharose, and many other enzymes. The object of all these enzymes is apparently to prepare the food for passing through the membrane into the protoplasm of the cells, where the final changes which liberate energy take place. The same processes occur with microörganisms but in a more simple manner. Surrounded by a liquid medium, they secrete enzymes; these dissolve certain insoluble foods which then diffuse through the cell wall to be decomposed further.

The food-preparing processes are all supposed to be simple hydrolytic processes. For some of these changes the chemical equations are well known. The hydrolyzation of starch to maltose by means of diastase is represented by the equation

$$2(C_6H_{10}O_5)_n + nH_2O = nC_{12}H_{22}O_{11}.$$

The splitting up of a fat molecule into glycerin and fatty acid is also a well-known process

$$\underset{\text{Tristearin}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3H_2O = \underset{\text{Glycerin}}{C_3H_5(OH)_3} + 3\underset{\text{Stearic acid}}{C_{18}H_{36}O_2}.$$

Proteolysis is not so well known and the general supposition that the first stages of protein degradation are hydrolytic is largely based upon analogies. Some of these enzymes which are secreted by the microbial cells act upon soluble compounds. *Invertase* decomposes saccharose into dextrose and levulose:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

Other disaccharides are hydrolyzed in the same way by other enzymes; glucosides are decomposed by *emulsin;* soluble proteins are changed to peptones. It is not necessary that the enzymes act upon the soluble compounds outside the cell since these compounds can diffuse into the cell; these enzymes are found only occasionally within the cell. It may be said, however, that the smaller molecules of the products of enzymic action diffuse more readily than the larger molecules of the original food compound.

Properties of Enzymes.—These secretions of cells are treated in a group by themselves because they differ distinctly in many respects from any other chemical substance. Probably the most notable difference may be discovered in the fact that their action does not follow the law of mass action which supposes that all substances reacting upon each other diminish in quantity. *Rennet* will coagulate many hundred times its weight of casein, and still the whey will contain rennet. Considering that part of the rennet is physically absorbed by the coagulum, the amount of rennet is found to be the same as before, though it has changed a comparatively enormous quantity of casein. The same is true with other enzymes. The enzyme is not destroyed by acting upon other substances. This exceptional quality furnishes a reason for treating enzymes as a separate group or apart from other chemical substances. But there are still other qualities which distinctly separate them from the well-known chemical bodies, and show at the same time their relation to proteins and toxins (page 179). One of these is their sensibility to such outside influences as will destroy life. Enzymes are inactivated by exposure to temperatures above 50° to 80°, and

can, like coagulated albumin, by no means be brought back to their original state. This temperature is very near the coagulating temperature of albumin. It is believed from this resemblance that enzymes are of an albuminous nature. Another similarity is the fact that both enzymes and albumins are precipitated by concentrated salt solutions. Enzymes can further be inactivated by poisons. The same substances which kill living cells, like formaldehyde, hydrocyanic acid, mercuric chloride, phenol, will also inactivate enzymes, though usually stronger solutions are required for the destruction of the enzyme than for killing the cell. It is the same with heat; a higher temperature is generally required to destroy the enzyme than to kill the cell which secreted it. Light will also affect enzymes considerably. The great similarity of enzymes and microörganisms in these respects, the similarity of their reactions and the extreme minuteness of the bacteria render it explicable why the chemists of eighty years ago could not determine the difference between microörganisms and enzymes, and called them both "ferments."

With the toxins, the enzymes have in common the great sensibility to heat, light, and chemicals. Both of these groups are resistant to drying to a limited extent. So far as body reactions are concerned these two groups seem to belong to one physiological group of compounds. When toxins are injected, the body responds by the production of antitoxins which inactivate the toxin. In the same way the body responds to enzymes by the production of anti-enzymes which prevent the action of the enzymes. It may be mentioned that against protein compounds, precipitins are produced by the body which precipitate only that protein which was injected. This "specific" action is also true with toxins and enzymes. The anti-body will inactivate only the specific kind of toxin or enzyme that was injected.

What an enzyme really is cannot be defined. An enzyme is known only by its reactions. Many chemists have tried to prepare pure enzymes by continuously dissolving and precipitating, by dialyzing and other means, but there are two great difficulties existing; there is no test for the purity of enzymes, and they lose in activity if treated with chemicals. The more they are freed from the protein bodies which always accompany them, the more sensitive they are to injurious influences. Mineral salts seem essential for their action, because con-

tinued dialyzing weakens the activity which can be restored only by adding salts.

ENZYMES OF FERMENTATION.—It has been demonstrated in the above paragraph that food is prepared for digestion or fermentation by enzymes. The final decomposition, the process which yields the energy for cell life, must take place within the cell.

The difference in importance of food preparation and fermentation may be illustrated by the example of *Rhizopus oryzæ*. This mold attacks starch, changes it, by means of diastase, to maltose, the maltose to dextrose, dextrose to alcohol and carbon dioxide. The mold grows well in a starch medium, without sugar; it grows equally well in maltose, and equally well, or better, in dextrose; it does not grow at all with alcohol and carbon dioxide. The last change, dextrose to alcohol, is absolutely necessary for this organism; it is the source of its life; the others are incidental processes, not absolutely necessary under all circumstances, in fact greatly suppressed if dextrose is given together with starch. The fermentation must take place in the cell; the preparation of food may take place in the cell or outside; it is not essential where it happens.

The investigations of recent years have demonstrated that fermentations also are caused by enzymes. It has been proved beyond doubt that in the alcoholic, lactic, acetic and urea fermentations the fermentation process may continue after the death of the fermenting cells. In the case of alcoholic fermentation, the fermenting agent was separated first by Buchner from the lacerated cells and was filtered through porcelain filters without losing its ability to act. This proves the enzyme-nature of the fermenting agent which, once being formed, remains and acts independent of the cell. These enzymes are called *zymases*. They remain within the cell as long as it is alive. They are much more sensitive to injurious influences than the above-mentioned food-preparing enzymes. Much skill and patience was required to demonstrate their independence of the living cell. After these enzymes were found in microörganisms, similar enzymes were discovered in the cells of higher plants and animals. Many of the biochemical changes taking place in the final dissociation of food within the cell are known to be the result of enzymic action; heretofore these reactions were believed to be a part of the life processes, inseparable from the living cell. Even some of the

oxidations and many reducing processes have been recognized as caused by enzymes, and it is quite probable that the whole process of intracellular food decomposition in all organisms is accomplished entirely by means of enzymes.

Classification of Enzymes

Since the chemical nature of enzymes and of their action is largely unknown, they can be classified only according to the compounds they act upon. It is possible, however, to distinguish between the following four groups: *Hydrolyzing*, *zymatic*, *oxidizing*, *reducing* enzymes. This definition is not quite exact, since the urea fermenting enzyme is also a hydrolyzing enzyme, and the acetic fermentation is caused by an oxidizing enzyme. The distinction between *endo-enzymes* (*intra-cellular*) and exo-enzymes (*secreted*) is not exact, either, since invertase and lactase are retained in the cells of some organisms and secreted by others.

The following classification is used in the further discussions:

I. *Hydrolytic Enzymes.*
   1. of carbohydrates: cellulase (cytase), diastase (ptyalin, amylase), invertase, lactase, maltase.
   2. of fats: lipase (steapsin).
   3. of proteins:
      (*a*) proteolytic (proteases): pepsin (peptase), trypsin (tryptase), erepsin (ereptase).
      (*b*) coagulating (coagulases): thrombase, rennet (chymosin).

II. *Zymases.*
   1. of carbohydrates: alcoholase, lactacidase.
   2. of other nitrogen-free bodies: vinegar-oxidase.
   3. of proteins: endo-tryptase, autolytic enzymes, amidase, urease.

III. *Oxidizing Enzymes.*
   Vinegar-oxidase, tyrosinase.

IV. *Reducing Enzymes.*
   Katalase, reductases of nitrates, sulphur, sulphites, telluric salts, methylene blue, litmus.

Several different names have been given to some of the enzymes; these are found in parenthesis in the above classification.

The general action of enzymes being explained in the preceding pages, it remains to describe more in detail the different enzymes of microbial origin.

Hydrolytic Enzymes

Enzymes of Carbohydrates.—Enzymes which decompose carbohydrates are very commonly found in nature, because carbohydrates onstitute a very extensive and common group of organic matter. y far the largest part of the dry plant consists of cellulose, starch nd sugar. To decompose them, enzymes are necessary. The chemal reaction of these enzymes is hydrolytic; in other words, the larger olecule is broken into smaller ones by the simple addition of water. hus, the cellulose-destroying enzyme, called *cellulase* or *cytase*, deomposes the cellulose into soluble sugars after the following formula:

$$C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$$

r, considering that the cellulose molecule is really many times $_6H_{10}O_5$, the formula will be more accurately written

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$$

hich indicates at the same time that one cellulose molecule gives any sugar molecules.

Cellulase is an enzyme which is quite difficult to obtain. Though must be produced by all the cellulose destroying molds and bacteria, xperiments have failed in some instances to prove its presence. It found in some wood destroying fungi and in some of the bacteria ausing the rot of vegetables. The organisms of certain plant diseases orce their way into the cell by dissolving the cellulose membrane by n enzyme, while certain molds are able to puncture the cell wall echanically.

Diastase, or amylase, is the starch-dissolving enzyme which is one f the most common enzymes in nature. It is found in all green plants, nd it forms during the sprouting of starchy seeds. Many molds nd a few bacteria produce this enzyme, while yeasts generally cannot ecompose starch for lack of diastase. Starch has the same formula s cellulose, and it is broken up into soluble sugars in the same way. Much attention has been paid to this process by the chemists, and it found that the process is a gradual one, giving first dextrins, and nally maltose ($C_{12}H_{22}O_{11}$). The hydrolysis of starch expressed in hemical symbols may be presented as follows:

$$2(C_6H_{10}O_5)_n + nH_2O = nC_{12}H_{22}O_{11}.$$

Starch Maltose

The disaccharides or double sugars, having the chemical formula $C_{12}H_{22}O_{11}$ are broken up into single sugars, monosaccharides, by the following process:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

The two molecules of $C_6H_{12}O_6$ are different with different sugars. If the disaccharide is saccharose, the two monosaccharide molecules are dextrose and levulose. Lactose will yield dextrose and galactose, and maltose will give two molecules of dextrose. For each of these sugars, there is a special enzyme which can hydrolyze only its particular sugar and none of the others; like a key, made for one lock, it will not open another lock. *Maltase* will split only maltose molecules, not lactose, while the *lactase* cannot attack the maltose. *Invertase* (or *sucrase*) will decompose nothing but saccharose. This decomposition of the complex sugars into the simple sugars was believed to be necessary because only sugars of the type $C_6H_{12}O_6$ can be fermented directly by the fermenting enzyme in the cell, be it an alcoholic or lactic or gassy fermentation. This explains why beer yeast cannot ferment lactose; it produces no lactase, and therefore cannot attack the lactose molecules; they would be easily attacked, if besides the yeast, some lactase were added. Certain lactic bacteria cannot ferment saccharose, because they do not form invertase. Recent experiments have shown that bacteria exist which ferment lactose and saccharose but not dextrose or levulose. An explanation for this cannot be given.

Invertase is, like diastase, a very common enzyme in green plants. It is also produced by most molds and yeasts, and bacteria. Maltase is not quite so common, and lactase is limited to a few species of microörganisms. A few organisms are known which do not secrete these enzymes but retain them within the cell. This is especially true of lactase, but is also known, in a few instances, of invertase. The enzyme can be obtained from the broken cells. Such enzymes are called *endo-enzymes*.

The decomposition of carbohydrates has been followed from the most complex representatives to the simplest ones, the monosaccharides. If these are decomposed further, the resulting product is no longer a carbohydrate. The simplest sugars are decomposed by zymases, inside the microbial cell, into compounds which are generally

called fermentation products; these may result from alcoholic, lactic, butyric fermentations or some other.

Emulsin is an enzyme which is able to hydrolyze glucosides. Glucosides occurring in plants are complex bodies which contain a sugar-radical. Emulsin splits glucosides liberating the sugar, usually dextrose. The typical example for emulsin action is the hydrolysis of amygdalin to hydrocyanic acid, benzaldehyde and dextrose.

$$\underset{\text{Amygdalin}}{C_{20}H_{27}O_{11}N} + 2H_2O = \underset{\text{Benzaldehyde}}{C_6H_5COH} + 2\underset{\text{Dextrose}}{C_6H_{12}O_6} + \underset{\text{Hydrocyanic acid}}{HCN}.$$

Emulsin is found in many molds and bacteria, and recently has been found in yeasts. Glucoside-splitting enzymes play an important rôle in the fermentations of coffee-beans, cocoa, mustard and indigo. In most of these fermentations, however, the emulsin is probably not formed by microörganisms, but by the plant, from which the fermenting material is derived.

Enzymes of Fats.—All the enzymes, acting on fat, decompose it in the same manner; the fat molecule takes up three molecules of water, breaking up into glycerin and three molecules of fatty acid, as indicated on page 168. It is possible that there are several fat-splitting enzymes, but the result of the cleavage process is always the same. The name formerly assigned to enzymes of fat is *steapsin*, but this term is now almost exclusively substituted by the more significant word *lipase*. Occassionally they are called lipolytic enzymes which expression is analogous to the proteolytic enzymes; in the same way, the term amylolytic enzyme is used for diastase.

Enzymes of Proteins.—The enzymes composing protein bodies, generally called proteolytic enzymes or *proteases*, have been known for nearly a century. Though the difficulty of analyzing protein bodies accurately prevents an absolute knowledge of proteolysis, much effort has been made to become acquainted with the very important group of enzymes which accomplish the digestion of protein food. Naturally most experimenting had been conducted with pepsin and trypsin of the animal body, accordingly these are better understood than others, and only little work has been done with microbial enzymes; but there is so far as can be determined little appreciable difference between the proteolytic enzymes obtained from different organisms, whether low or high in the plant or animal world, consequently many experi-

ences with animal pepsin and trypsin can be applied to microbial enzymes.

The specific chemical action of these enzymes is referable to hydrolysis; the large protein molecule is broken up into smaller molecules by addition of water. Various proteolytic enzymes differ in the extent of decomposition. While some, like pepsin, produce mainly peptones, trypsin is able to split protein to amino-acids and even to ammonia. Mavrojannis tested for the intensity of gelatin decomposition with formaldehyde. The peptones of gelatin will solidify with formaldehyde while amino-acids are not affected.

Proteolytic enzymes were first divided into two groups: *pepsins*, which act best in slightly acid solutions, and *trypsins*, which act best in slightly alkaline media. The names are derived from pepsin (peptase) the proteolytic enzyme of the animal stomach, and from trypsin (tryptase) which is found in the small intestine of animals. This classification cannot be used for the enzymes of microörganisms because there is no definite line established by the acidity. Some enzymes work in either acid or alkaline media equally well, preferring a neutral reaction. Enzymes should be classified according to the substances they act upon or perhaps according to the nature of the products resulting from the fermentation. This would bring pepsin and trypsin into one class, both acting upon protein bodies as such; they, however, differ in the intensity of action as shown by their products, the pepsin forming mainly peptones, the trypsin carrying on the decomposition as far as amino-acids and traces of ammonia. Another class recently recognized is *ereptase* (erepsin) which cannot decompose protein, but readily attacks peptones, decomposing them much in the same way as trypsin. Pepsin, trypsin and erepsin do not break up amino-compounds.

The presence of proteolytic enzymes in microörganisms is readily tested by cultivation on nutrient gelatin. The proteolytic enzyme secreted by the cells will liquefy the gelatin. Generally, an organism that liquefies the gelatin will also decompose the casein of milk and the protein of blood serum. There are some exceptions, however, as is shown in the following table, after Frost and McCampbell. A + sign means proteolysis, a − sign means no action.

Organism	Milk		Gelatin	Serum	Egg album.	Fibrin
	Coag.	Digest.				
Bact. anthracis	+	+	+	−	+	+
Microspira comma	+	+	+	+	+	+
M. pyogenes var. aureus	+	+	+	−	−	−
Pseudomonas pyocyanea	+	+	+	+	+	−
B. violaceus	−	−	+	−	−	−
B. mycoides	+	+	+	−	+	−
B. prodigiosus	−	+	+	+	+	+
Aspergillus niger	+	+	−	−	−	−
Aspergillus oryzæ	−	+	+	+	+	−

Apparently not all organisms which liquefy gelatin are able to decompose egg albumin; we must conclude that the enzyme liquefying gelatin is different from the proteolytic enzyme dissolving egg-white.

Coagulating Enzymes.—The blood-clotting enzyme (*thrombase*) does not occur in microörganisms. *Rennet*, however, is found in many species. Rennet is extracted from the stomach of calves and pigs and used to set the curd in milk for cheese making. The enzyme acts upon the casein in milk, decomposing it into paracasein and some soluble protein. The time of coagulation depends upon the temperature of the milk and the concentration of the rennet. This coagulation of milk is quite different from the acid curd, where the insoluble casein is precipitated by the acid. If enough acid is added, the milk curdles immediately; if there is not enough acid, there will be no curd, not even after a long time. An acid curd can be brought back to the original state by an addition of alkali, while a rennet curd by no means can be changed back to casein. Rennet-forming bacteria are found in milk and dairy products, in soil and other habitats. They will coagulate milk without causing any appreciable increase of acidity. They all seem to digest the curd after it is formed (see the above table). The relation between proteolytic and rennet enzymes will be discussed in a later chapter.

Rennet is sometimes called chymosin; the Society of American Bacteriologists uses the German word "*lab*."

Zymases

The zymases are the agents which furnish the energy for cell life by causing fermentative decompositions. As has been stated before, the processes which provide for energy must take place inside of the cell. Consequently, all fermenting enzymes are endo-enzymes. The difference between the soluble enzymes and the endo-enzymes is very plainly shown in the following table, giving the energy liberated by the various enzymes by acting upon 1 g. of substance.

Energy Liberated from 1 g. of Substance

Soluble Enzymes		Endo-enzymes	
Pepsin, trypsin	0 calories	Lactacidase	80 calories
Lipase	4 calories	Alcoholase	120 calories
Maltase, invertase	10 calories	Urease	230 calories
Lactase	23 calories	Vinegar-oxidase	2,500 calories

The microbial cell does not lose much energy by the activity of the soluble enzymes outside of the cell, because their energy yield is insignificant.

The first zymase known was *urease*, the enzyme which changes urea to ammonium carbonate. The actual investigation of the zymases did not start until Buchner had demonstrated that yeast can be ground with infusorial earth until all cells are lacerated, and then can be pressed and the juice filtered without losing the power of alcoholic fermentation. Such fermentation cannot be due to anything but a soluble compound of the yeast cell. Thus the *alcoholase* was discovered. It was found later that yeast may be killed by alcohol, ether or acetone without losing its fermenting power.

This last method was applied later to lactic bacteria, and it was proved that the lactic acid is also produced by an enzyme, *lactacidase*. It is possible to kill the lactic bacteria cells so that they do not multiply but still continue to form acid. It seems quite probable that other fermentations of carbohydrates, like the butyric and the gassy fermentations, are really due to enzymes. It is very difficult to give the experimental proof, however. These enzymes are so unstable that it requires much experience to separate them from the cell, and it is also quite difficult to obtain bacteria in quantities large enough for such experiments.

The vinegar oxidase is an enzyme which remains in the cell of the acetic bacterium, oxidizing alcohol to acetic acid. Its independence of the living cell has been demonstrated by killing the cells with acetone.

The PROTEOLYTIC ENDO-ENZYMES of yeasts, only, have been studied extensively. That such enzymes exist is recognized by the observation that certain microörganisms do not liquefy the gelatin until after they are dead and the proteolytic enzymes diffuse out through the deteriorating cell membranes. That yeast in the absence of sugar loses in weight, and that leucin and other cleavage-products of protein are formed, was the first indication of a proteolytic process in the yeast cells. By pressing the juice out of the ground yeast cells, a liquid is obtained which liquefies gelatin, digests casein, albumin and fibrin. The living yeast cell does not attack these compounds, because they cannot diffuse into the cell and the enzyme cannot diffuse out. The proteolytic endo-enzyme of yeast is called *endo-tryptase.* Its object is apparently the regulation of the protein-content of the cell and perhaps it has some bearing on the formation of cell plasma. The possible relation between enzymes and growth is discussed in a following sub-chapter.

If yeast is mixed with a weak antiseptic (chloroform, toluol) the proteolytic process takes place quite rapidly. This process is called *autolysis* (self-digestion). Similar autolytic enzymes are found in other microörganisms. Autolysis is a well-known process in the higher animals. To this is due the ripening of meat.

Proteolytic endo-enzymes must be expected in all microörganisms which depend upon protein as food material only. These organisms will secrete certain enzymes which decompose the insoluble protein into bodies which diffuse easily into the cell. Here, proteolytic endo-enzymes further decompose these products. Such an endo-enzyme is the *amidase* discovered by Shibata in the mycelium of *Aspergillus niger* which forms ammonia from urea, acetamid, oxamid, biuret. *Endo-erepsin* and amidase were also found in *Penicillium camemberti* by Dox.

Similar to these proteolytic enzymes is the *urease* which is formed in large quantities in the so-called urea bacteria, but it is also present in the mycelium of some molds. An endo-enzyme, splitting hippuric acid into benzoic acid and glycocoll, is found in the mycelium of a few molds.

Oxidizing Enzymes

The most typical example of an oxidizing enzyme is the *vinegar-oxidase*, because its chemical action is well known. Most of the *oxidases* known act upon complex organic compounds, changing them to colored bodies. Such an oxidase is the *tyrosinase* which forms a black, insoluble compound in tyrosin solutions. It is produced by several bacteria, especially by chromogens, and its application in testing for small quantities of tyrosin has been suggested. A number of oxidases are known to act upon the leuco-bodies of certain organic dye-compounds, as aloin, guaiac, phenolphthalein, and others. Hydrochinon is oxidized by the dead cells of a few molds. Strange seems the oxidation of potassium iodide to iodine by the *endo-oxidase* of a mold. Many other oxidations are supposed to be of enzymic nature, but their independence of the living cell has not been proved.

Many higher organisms are known to contain oxidases, the best studied are those of certain mushrooms which change the white mushroom meat into a bluish or brownish color as soon as it is exposed to the air. Oxidases are very common in most of the tissues of higher animals.

Reducing Enzymes

Among the *reductases*, one enzyme stands apart from all the others, that is the *katalase* or *peroxidase* which reduces the hydrogen peroxide to water by liberation of oxygen.

$$H_2O_2 + \text{katalase} = H_2O + O.$$

Katalase is one of the most commonly found enzymes; it is formed by practically all plants and all animals and is contained by all but a few bacteria. Among these exceptions is the *Strept. lacticus*. The absence of katalase in this species has been recommended as a diagnostic test. It is possible that this enzyme is necessary for intra-cellular oxidations.

A number of other *reductases* are known. Nearly all of the reductions mentioned in the paragraph on the products of mineral decomposition are proved to be of enzymic nature; these processes will take place after the cell is killed by a disinfectant or is ground to pieces. This can be readily demonstrated by lacerating the cells

with quartz sand. They will then reduce nitrates to nitrites, sulphur to hydrogen sulphide. The decolorization of litmus, methylene blue, indigo, and other organic dyes is due in microbial cultures to enzymes which are almost exclusively endo-enzymes.

Additional Remarks on the Relation of Cells and Enzymes

Enzymes are produced only by living cells. After they are once formed, they act like chemical compounds, independent of the cell which produces them. Even the endo-enzymes follow only the law of enzyme-action and are not influenced by the cell which contains them. The enzymes are mostly influenced by their own products, and when a certain yeast ceases to ferment sugar at the concentration of 8.5 per cent of alcohol, this means that the alcoholase of this yeast cannot tolerate more than 8.5 per cent of alcohol. The inability of the cell to regulate enzymic action may account for the fact that often a culture produces an amount of fermentation products sufficient to kill all cells. This is observed in the lactic, acetic and alcoholic fermentations, and, perhaps, occurs in many others.

Probably, all cells produce several enzymes. Microörganisms feeding upon various foods must form various enzymes. Frequently several enzymes are necessary for the decomposition of one compound. *Rhizopus oryzæ* uses three enzymes in order to form alcohol from starch, first the diastase to change starch to maltose, then maltase to change maltose to dextrose and finally alcoholase to change dextrose to alcohol and carbon dioxide. The number of enzymes formed by certain microörganisms is surprising. *Aspergillus niger* has the reputation of forming almost all enzymes which have ever been found in microörganisms. *Penicillium camemberti* produces (after Dox) erepsin, nuclease, amidase, lipase, emulsin, amylase, inulase, raffinase, invertase, maltase and lactase. It has been believed for a long time that certain enzymes are regular products of the cell while others are formed only if the substance upon which they act is present. According to Dox's investigations with *Penicillium camemberti*, there is no evidence that enzymes not normally formed by the organism in demonstrable quantities can be developed by special methods of nutrition. The addition of a particular food compound does not develop an entirely new enzyme, but stimu-

lates the production of the corresponding enzyme which is normally formed, although in small amounts, under all conditions.

Theory of Katabolism

Regarding katabolism as the sum of all destructive processes of the living cell substance, *i.e.*, of the protoplasm, and considering the cell substance to be decomposed and renewed constantly as long as the cell is performing the normal functions of life, there must be a renovating and a destructive process continuously going on in the protoplasmic molecules. If the food supply ceases, anabolism ceases with it, but it has been demonstrated that katabolism may continue just the same for some time. By this method, the products of katabolism can be obtained separate from the products of food digestion which would obscure the results of experiment on katabolism in normally fed cells.

It is difficult to determine to what extent katabolism is controlled by *endo-enzymes*, the so-called *autolytic enzymes*, which have been mentioned in the above paragraph. Unquestionably, the katabolic processes are similar to enzyme processes, since katabolism is checked by heat or poison just like enzyme processes.

Theory of Anabolism

Anabolism and Intra-cellular Enzymes.—All changes discussed in the previous chapters are processes in which organic or inorganic compounds are broken up to smaller molecules. These processes are exothermic, *i.e.*, liberating heat or energy in other forms. The opposite is true of the anabolic processes which build up complex molecules from simple compounds. These synthetic processes are endothermic, absorbing heat or other energy. Growth is the typical manifestation of anabolism. It is the formation of new cells from dead organic or inorganic matter, and it means the formation of all the compounds necessary for cell life. Of all the substances found in the cell, practically none are contained in the food, and it is wonderful that in such a small unit as a microbial cell, there are contained the powers of making protoplasm, enzymes, nuclear bodies, chromatin bodies, the substance of the cell wall and probably many other unknown

ompounds. All these complex substances are generally made from imple food compounds as amino-acids, carbohydrates and others.

These synthetic processes of the cell will, like most endothermic rocesses, take place only if energy is provided. This condition is sually fulfilled in the living cell, due to the fermenting processes oing on continuously. There is a strange interaction between nabolism and intra-cellular fermentation proceeding in the pro- oplasm and this linking together of destructive and constructive eaction is the basis of life processes. The life processes decompose ertain substances, the energy liberated allows the formation of proto- lasm, which again liberates energy. Thus a continuous formation of rotoplasm is secured.

An explanation of anabolism based upon chemical experiments is ot possible at the present time. In the study of intra-cellular destruc- ion it is possible to trace most processes back to enzymic action. here our knowledge ceases because the nature and mode of action f enzymes is unknown. In the study of anabolism our knowledge as not even progressed so far. The most promising explanation at resent is based upon the *reversibility* of enzymic action.

Reversibility of Enzymic Action.—Chemical reactions be- tween organic compounds proceed quite rapidly at first, then become slower and slower until the reaction stops entirely. The reaction is not complete at the time it reaches an equilibrium. If the equilib- rium is disturbed by adding more of the reagents, the process will continue. If, however, the products of reaction are added, the reverse process will take place. Reactions between organic compounds can proceed either way, depending upon the relative concentrations of the reacting substances. The standard example is esterification. As- cetic acid plus alcohol gives ester plus water,

$$\underset{\text{Acetic acid}}{CH_3COOH} + \underset{\text{Alcohol}}{CH_3CH_2OH} \rightleftarrows \underset{\text{Ester}}{CH_3COOCH_2CH_3} + H_2O.$$

The process goes to a certain equilibrium and stops. If ester is mixed with water, it gives acid plus alcohol, until the same equilibrium is reached. If acid and alcohol are added to a system in equilibrium, more ester will be formed. If ester is added, more alcohol and acetic acid will be formed. The same is true with enzymes, at least with some enzymes. Maltase will decompose maltose into two molecules of dextrose. In a concentrated solution of dextrose, however, maltase

will form maltose, or a similar sugar, isomaltose. Lipase is able to produce fat from glycerin and fatty acids. A solution of albumose with trypsin or pepsin gives a precipitate of a body which is more complex than albumose and which gives the protein reactions. It is believed by many physiologists that pepsin and rennet are the same body. Under certain conditions, it has a dissolving power, under other conditions it has the power to coagulate.

The reversibility of enzymic action has given rise to much speculation about assimilation and growth. It seems reasonable to suppose that the cell forms its protoplasm from amino-acids by the reversed action of proteolytic enzymes. In the same way, cellulose may be formed from dextrose, fat from glycerin and fatty acids. Nearly all phases of growth can be accounted for in this way. This is nothing but theoretical speculation, and the only fact to support it is the reversibility of certain enzymes. The conditions under which chemical reactions take place inside of the cell are very largely unknown. There are so many processes going on at the same time that it is absolutely impossible at the present time to obtain a perfect understanding of all these reactions. Thus, our knowledge of growth is largely based upon analogy and speculation.

DIVISION II

PHYSICAL INFLUENCES

CHAPTER I

MOISTURE

Moisture may be called the most important factor of life. Not nly bacteria, but every microscopic and macroscopic being requires a onsiderable amount of moisture. Living organisms contain on the verage between 70 per cent and 90 per cent of water, and only 10 per ent to 30 per cent of solid matter. Microörganisms which live ntirely submerged in liquids need water not only within but without ne cells. Bacteria, yeasts, molds, and some protozoa obtain their food y diffusion through the cell-membrane; their food-substances must e soluble and dissolved. No other liquid can take the place of water.

The amount of water required by microörganisms cannot be stated riefly. Several factors have to be taken into consideration, as the smotic pressure, the insoluble and the colloidal substances, the species f organisms, temperature, and perhaps others.

OSMOTIC PRESSURE.—In the organic world we find very commonly nembranes which will allow water to pass through but retain some ompounds dissolved in the water. Such so-called semi-permeable nembranes are found surrounding the protoplasm of cells. They are ot the cell wall, but separate the protoplasm from the cell wall. imilar properties are found in parchment paper, pig's bladder, and ther organic membranes.

If a salt solution is poured in water, the two liquids will mix in a hort time and soon every smallest portion of the mixture will have the ame concentration. If a salt solution and water are separated by a nembrane which does not allow the salt to pass, the water will go hrough the membrane toward the salt with a certain amount of ressure. This pressure depends upon the nature of the dissolved ubstance as well as upon its concentration.

The pressure increases in direct ratio with the number of molecules in solution. Therefore, a compound with large molecules (cane sugar) will produce a lower osmotic pressure than one with small molecular weight (glycerin) if we compare solutions of equal concentration. The osmotic pressure of protein, starch and peptone solutions can be measured only with the finest instruments, while the pressure of a 30 per cent dextrose solution is 22 atmospheres.*

Plasmolysis.—If a cell is brought into a strong solution of a substance which cannot pass the plasma-membrane, this substance will cause an osmotic pressure and the concentration in the cell being lower than in the medium, the water will pass out from the cell until the pressure inside and outside is the same. This causes a shrinking of the protoplasm, while the rigid cell wall keeps its shape. Such plasmolyzed organisms are illustrated in Fig. 67, page 87.

While plasmolysis is easily demonstrated with the cells of higher plants, microörganisms do not show it so readily. In fact, many bacteria, like *B. subtilis*, *Bact. anthracis*, cannot be plasmolyzed by any concentration of salt in solution. Others, as *B. coli*, *B. fluorescens*, react promptly. But even though many are killed, the rest recover from plasmolysis after a few hours, and appear normal. This indicates that the salt passes slowly through the plasma-membrane and thus increases the pressure inside the cell until finally the inside and outside pressure are the same again.

The fact that many microörganisms show no plasmolysis whatever is explained in the same way. These organisms probably have plasma-membranes so constructed that the salts diffuse through nearly as fast as the water. An absolute exclusion of all soluble substances by the membrane is impossible since the food can get into the cell only by diffusion through the membrane.

The resistance of various microörganisms against concentrated solutions depends upon the organism as well as upon the dissolved substance. The sodium and potassium salts of the common mineral acids act upon a culture nearly in proportion to their osmotic pressure, but the potassium salts always retard growth a little less than the sodium salts. The effect of salts upon microörganisms is therefore not due to the osmotic pressure only; the chemical constitution of the salts also plays an important rôle.

* One atmosphere equals the pressure of 1 kg. per square centimeter or about 15 pounds per square inch.

The different functions of life are influenced in different degrees by oncentrated solutions. Some bacteria will multiply but not form pores in salt solutions. Molds will sometimes show a good growth in oncentrated sugar solutions but fail to produce spores. *Bact. anthracis* oses its virulence in sea water. Often, the form of microörganisms is ffected by concentrated solutions. Some bacteria grow more spherical, thers become elongated or distorted. The deforming influence is not ue to the osmotic pressure only, but depends mainly upon the chemical haracter of the salt; magnesium salts especially have a tendency to roduce such involution forms.

Salt and Sugar Solutions.—Most experiments on the influence of oncentrated solutions have been carried on with sodium chloride, be-ause of its wide application in the preservation of foods. Most micro-rganisms, especially the rod-shaped bacteria, are suppressed by a salt oncentration of 8 to 10 per cent. At 15 per cent only few cocci develop lowly, while some species of *Torulæ* grow without a very noticeable re-ardation. Above 20 per cent the *Torulæ* are practically the only rganisms which can develop. They are, therefore, found in all food roducts which are preserved by salt, as salted pork, beef, fish, butter, nd pickles, often in nearly a pure culture. It seems that they are asily overpowered by other organisms in the absence of salt, but in salted food, this competition is eliminated.

The selective influence of salt is used in some fermented products to prevent undesirable fermentations. This is true in sauerkraut and brine pickles, where the desirable bacteria can grow in the presence of salt while the undesirable ones are kept away. Possibly the salting of butter has the same effects.

Another compound of great practical importance is cane sugar, which is the standard preservative for fruits and condensed milk. Its action has been studied mainly upon molds. Theoretically, dextrose should be expected to have twice as strong a preserving action as saccharose because it has only half the molecular weight and consequently produces twice as strong an osmotic pressure in the same percentage of concentration. Its preserving effect is indeed a little higher than that of saccharose, but the proportion is not nearly 1:2. The common molds are extremely resistant to strong sugar solutions, about 60 to 70 per cent of cane sugar seems to be the limit of growth for *Penicillium* and *Aspergillus* species. Yeasts can also grow and ferment in very con-

centrated solutions while bacteria in general do not tolerate solutions higher than 15 to 40 per cent, though many exceptions are known.

Colloidal Solutions.—In order to determine the amount of water which is absolutely necessary for microbial proliferation, only such media can be used which do not cause osmotic pressure. If *B. prodigiosus* does not develop in a 10 per cent salt solution, this is not due to lack of moisture, because the same bacillus will grow in a 30 per cent sugar solution which contains 20 per cent less moisture. Another factor besides the water content enters, which can be avoided only in solutions without osmotic pressure.

A few substances are known to give such solutions, namely, colloidal bodies which have a very large molecular weight. Their osmotic pressure even in very concentrated solutions would not be high enough to interfere with microbial growth. Among these colloidal bodies are found egg albumin, gelatin, peptones, all protein substances; also starch, dextrin and gum arabic among the carbohydrates. None of these substances has a retarding influence upon bacteria; some of them can be mixed with water in all proportions; consequently, they are the ideal medium to test the water requirements of microörganisms.

Experiments carried on with gelatin, powdered meat, crackers, bread and potato, vary but little in results. A few bacteria cannot grow in a medium with only 60 per cent water, but most organisms develop slowly even with 50 per cent water and some may be able to develop with only 40 per cent. Molds can grow very scantily in even more concentrated media. Protozoa probably have to have a more diluted medium for their development though no experiments bearing upon their water requirements are known to the author.

The fact that in a colloidal solution growth will cease if the moisture is below 30 to 40 per cent does not necessarily indicate the conclusion that any substance with less than 30 per cent water cannot be decomposed. The above statement refers only to solutions, while in natural media as dried foods or soil, a combination of solid and dissolved substances is involved. Butter is an excellent medium for many bacteria, yeasts, and molds, though it contains only 12 to 15 per cent of moisture. If butter fat were soluble in water, the concentration of 85 parts of solid in 15 parts of liquid would certainly prevent any growth whatever, but fat is insoluble, and the fat particles do not interfere at all with the growth of microörganisms in the droplets of buttermilk

distributed all through the butter. The concentration in these small droplets is the deciding factor. If the growth of microörganisms in butter is to be prevented by salt, it is unnecessary to give any attention to the fat; the bacteria live only in the water and not in the fat globules. In adding 3 per cent of salt to a butter with 15 per cent of moisture, a brine of 3 parts of salt in 15 parts of water is produced; in other words, a 20 per cent brine, because salt does not dissolve in the fat. Similar considerations will come up in the preservation of fruit, vegetables, meat, milk, and other food substances by drying or condensation.

DESICCATION.—Microörganisms do not die immediately after the removal of the water, and they do not die all at once after a given time. Death through drying is a slow and regular process. Paul and his associates founded that the number of bacteria dying in the unit of time is, under constant conditions, proportional to the number surviving. If we had 1,000,000 cells per gram in the beginning, and the death rate were 90 per cent per day, there would be, at the end of each day, 10 per cent of the original number surviving. This would give the following numbers for one week:

Beginning	1,000,000 cells per gram.
After 1 day	100,000 cells per gram.
After 2 days	10,000 cells per gram.
After 3 days	1,000 cells per gram.
After 4 days	100 cells per gram.
After 5 days	10 cells per gram.
After 6 days	1 cell per gram.
After 7 days	0.1 cell per gram.

This table shows graphically the mode of death of dried bacteria. The number of cells approaches zero without ever (at least theoretically) reaching it. From one cell per gram after six days we do not come to 0 on the seventh, but to one cell in 10 g. and on the eighth day one cell in 100 g. The total number dying in the first day is much larger than that dying on the sixth day, but the rate is constant, 90 per cent of the number surviving. This regularity has been found with bacteria dying from various causes, and it is commonly compared with the simplest chemical processes, the monomolecular reactions.

Paul and his associates found further, that the death through drying is caused by an oxidation process; in pure oxygen bacteria died much faster. The poisonous effect of oxygen upon moist bacteria has already been pointed out on page 156.

Most resistant to drying are the spores of bacteria; mold spores, too, show considerable resistance, while some bacteria, *e.g.*, *B. carotarum* and *Ps. radicicola*, are readily killed.

The resistance of microörganisms is influenced greatly by the medium on which they are placed for drying. Hansen found that yeast cells dried on cotton were still alive after two to three years, while if dried on platinum wire some died in five days and others lived as long as 100 days. Compressed beer-yeast mixed and dried with powdered charcoal kept as long as ten years; *Ps. radicicola* dried on a cover-glass or filter-paper died within twenty-four hours; on seeds, this same organism was still alive after fourteen days and in the dried nodules of legumes a few cells were able to reproduce after more than two years. Soil containing an average number of 17,000,000 bacteria per gram was dried for two years; the total number of organisms averaged then 3,250,000, 20 per cent of the bacteria, therefore, could resist desiccation. Dried cultures of microörganisms are commonly sold for several purposes, as dairy-starters and the so-called "magic yeast" and "yeast foam" used for bread-making. Such cultures are dried on milk, sugar, starch, flour or similar porous and absorbing material. Starters are usually guaranteed only for a certain length of time, from one to twelve months. The advantage of the dry culture is its better keeping qualities. Liquid cultures produce substances harmful to themselves, and die rapidly after a short time, while the dry cultures show little change.

The resistance of pathogenic bacteria to desiccation is of considerable importance in the spreading of contagious diseases. Many pathogenic bacteria die after desiccation of a few hours to a few days, and spreading of such diseases by dust is highly improbable. Protozoa of soil decrease in number by drying, but all are not killed.

CHAPTER II

INFLUENCE OF TEMPERATURE

Temperature, as well as moisture, is one of the most important factors of life. It is so important that the most highly developed animals protect themselves by a very complicated mechanism of regulation against changes of temperature; the life processes of such animals will take place at a temperature nearly constant from birth to death. This causes the metabolism of warm-blooded animals to be different from that of all other organisms. The metabolism of the warm-blooded animals takes place at a constant temperature. The required amount of food is constant except for the part that is used for heating the body; at lower temperatures, more heat-producing material is used and the result is that warm-blooded animals require more food at lower temperature. All other organisms, reptiles as well as bacteria, have the temperature of their environment and the decrease of temperature will decrease the intensity of metabolism as it retards any other chemical process. The lower the temperature, the less food is required by all lower organisms.

There are, of course, limits to the favorable influence of high temperatures. Growth and metabolism of microörganisms will increase with rising temperature to a certain point, called the *optimum temperature*, and beyond this point the rate of growth will fall off rapidly and soon cease entirely. The highest temperature at which growth can take place is called the *maximum temperature*. Correspondingly, the *minimum temperature* of an organism is the lowest point at which growth can take place.

THE OPTIMUM TEMPERATURE which allows the fastest growth will be quite different for different species. Groups of bacteria are known which develop only at very high temperatures and others for which room temperature is too high. The temperature requirement is largely dependent upon the natural habitat of the organisms. The bacteria of

the polar sea and of a lagoon near the equator will very probably have different optimum temperatures because of the acclimatization and selection which has been taking place for centuries.

The great majority of bacteria and related organisms, in fact of all living organisms, except in a few instances, has its optimum temperature between 20° and 40°. The optimum temperature of an organism is generally somewhat higher than the average temperature of its natural habitat.

The following table shows the data obtained for a few microörganisms.

Species	Temperatures		
	Minimum	Optimum	Maximum
Penicillium glaucum	1.5°	25°–27°	31°–36°
Aspergillus niger	7°–10°	33°–37°	40°–43°
Saccharomyces cerevisiæ I	1°–3°	28°–30°	40°
Saccharomyces pasteurianus I	0.5°	25°–30°	34°
Bacterium phosphoreum	below 0°	16°–18°	28°
Bacillus subtilis	6°	30°	50°
Bacterium anthracis	10°	30°–37°	43°
Bacterium ludwigii	50°	55°–57°	80°

THE MINIMUM TEMPERATURE or the lowest limit of growth is usually farther from the optimum than the maximum temperature. It will vary with the organisms just as do the other cardinal points. But there is a natural limit drawn by the freezing-point of the nutrient liquid. Not all organisms can grow at such low temperatures, in fact the greater number does not develop below 6° to 10°. Those that can grow at the freezing-point will be inhibited by the solidification of the water in the nutrient medium, for if the water is frozen, food cannot diffuse into the cells and therefore, all life processes are checked. If freezing is prevented by adding salts or other soluble substances which lower the freezing-point, growth may continue even below 0°. Milk freezes at about − 0.5°. Bacteria are found to multiply in it as long as it is not entirely solid. A certain yeast multiplied slowly in salted butter kept at about − 6°.

The number of microörganisms that developed at the freezing-point was found to be:

In 1 c.c. of market milk, up to 1,000 germs.
In 1 c.c. of sewage, up to 2,000 germs.
In 1 g. of garden soil, up to 14,000 germs.

THE MAXIMUM TEMPERATURE is usually about 10° to 15° higher than the optimum. The development of microörganisms above the optimum temperature is not quite normal; there is a great tendency toward involution forms. The mycelium of molds grown near the maximum temperature appears unhealthy and pathogenic bacteria lose part of their virulence. This loss of virulence is made use of in the preparation of attenuated cultures for vaccines.

The maximum temperature varies with different species of bacteria. Most bacteria do not grow above 45°, but with some of the maximum temperature is considerably lower. *Bact. phosphoreum* dies if exposed for a few hours at 30°; others may require still lower temperatures. The average organisms found in water, soil, milk, and the body, which have their optimum near 30° to 38°, do not grow higher than about 45°. There are very noticeable exceptions to these, such as the physiological group known as thermophilic bacteria.

These extraordinary organisms have their maximum between 70° and 80°, a temperature which coagulates albumin. Corresponding to the high maximum the thermophiles have a very high optimum, and the minimum lies with most of these species above 30°. These organisms are found in soil, sewage, ensilage and occasionally in milk. They find the temperature suitable for their life only under extraordinary circumstances, as in fermenting manure piles, in silos, in self-heating hay and similar organic material that develops a high temperature by fermentation. Some hot springs have a very remarkable flora of thermophilic bacteria.

The range of temperature within which growth is possible, is very uniformly 35° to 45°; the starting points and end-points of this range vary greatly, while the total range is quite constant, except for some bacteria adapted to special conditions, such as some pathogenic bacteria. The temperature relations of bacteria can be shown graphically by using as ordinate the rate of growth, as abscissa the temperature.

Biological Significance of the Cardinal Points of Temperature.—The importance of the temperature requirements of certain organisms to the rôle they play in nature can be illustrated by a few examples. Most molds cannot cause disease in man and warm-blooded animals because their maximum temperature is below the body temperature. Exceptions are some *Aspergilli* and *Mucorineæ*. Pathogenic microörganisms must have their optimum temperature coincide with that of their host.

Organic substances may undergo a different change at different temperatures. The biochemical changes in soil may not be the same in northern Canada and near the Gulf of Mexico. Even the warm and cold season of the same climate is apt to change not only the rate of decomposition but possibly the products. Perhaps the most striking example in this respect is the decomposition of ordinary market milk kept at different temperatures. Such milk contains a great variety of microörganisms; at various temperatures different types will predominate, while the remainder are retarded or inhibited by unfavorable temperature conditions and by the products of the dominant type of bacteria. If milk is kept at about the freezing-point, only a few organisms will develop slowly, but after a certain time their number will increase to many million cells per c.c. There is, however, no apparent change; no acid or deterioration can be discovered by the taste though chemical analysis proves the presence of hydrogen sulphide and ammonia. Between 15° and 25°, milk will sour in about thirty-six to forty-eight hours, giving a firm curd of an agreeable flavor without whey or gas; later *Oidium lactis* destroying the acid develops on the surface. Near body temperature the milk will lopper in twenty-four hours, the curd is usually contracted, a large quantity of whey is extruded, and much gas is produced by *Bact. aerogenes* and *B. coli*. The odor is disagreeable and later butyric acid is produced; eventually the lactic acid increases further by the action of *Bact. bulgaricum*. If kept above 50° the milk either keeps permanently, or a decomposition by thermophilic bacteria begins which is either an acid fermentation followed by digestion or a complete putrefaction, depending upon the species of thermophilic organism that happens to be in the milk sample. Thus there can be induced in the same substance, containing the same organisms at the start, four entirely different types of decomposition merely by the difference of temperature.

This indicates the importance of temperature regulation in the fermentation industries. Even pure cultures may give different products working at different temperatures. Cream ripened with a pure culture starter at too high a temperature will have a sharp acid flavor. The cold curing of cheese has become a very common practice because of the much improved flavor. Bioletti claims that the value of the dry California wines would be doubled if the fermentation were carried on generally at a lower temperature.

END-POINT OF FERMENTATION.—Another question is the relation between the end-point of fermentation and the temperature. Of the few data existing, many indicate that at a lower temperature the final fermentation goes farther than at a higher temperature. Müller-Thurgau found that under exactly the same conditions with the temperature as the only varying factor the following final amounts of alcohol were produced by a pure culture of yeast:

At 36°..... 3.8 per cent alcohol.
At 27°..... 7.5 per cent alcohol.
At 18°..... 8.8 per cent alcohol.
At 9°..... 9.5 per cent alcohol.

Concerning the lactic fermentation some investigators find no difference in the end-point, while others obtained results similar to the results with alcohol. With three strains of *Bact. lactis acidi* were obtained after thirty-four days, by C. W. Brown:

	A	B	C	
At 37°	0.89 per cent	0.87 per cent	0.60 per cent	of lactic acid.
At 30°	1.00 per cent	0.96 per cent	0.81 per cent	of lactic acid.
At 18°	1.08 per cent	1.06 per cent	0.88 per cent	of lactic acid.
At 6°	0.70 per cent	0.73 per cent	0.62 per cent	of lactic acid.

These results are quite logical and perhaps can be explained by the recognized experience that all products of fermentation tend to check the process of fermentation, and that any chemical product or substance acts the more vigorously upon any life process the higher the temperature. The same amount of alcohol that will still allow a slow fermentation at 10° may check the fermentation entirely at 20°. Naturally the rate of fermentation in the beginning will be higher at the higher temperature but the end-point is lower. The end-point of the lactic cultures A, B, and C at 6° is probably not final, because

thirty-four days is a short time of growth at so low a temperature Above the optimum, the rate of decomposition will decrease rapidly with the rising temperature and the end-point will also be lower.

FREEZING.—The discussion of the relation of temperature to microörganisms has so far considered only the temperatures within the limits of growth. However, the temperatures below the minimum and above the maximum are also of greatest importance. If bacteria are cooled below their minimum temperature they do not die immediately. They remain alive in a dormant condition ready to multiply as soon as the temperature rises. Even the freezing of a liquid will not kill them immediately. Of course, they cannot multiply in ice because they have no water, consequently no food, and they cannot thaw the ice to get their water and food for lack of body temperature of their own. As long as liquids are frozen solid the bacteria in them will remain dormant much like dried organisms, and like them their number will decrease very slowly. An example is given in the following table relevant to the number of bacteria in frozen milk (after Bischoff). The decrease in numbers is not very uniform, since there are many different bacteria in milk, but the general tendency is the same as in the dried bacteria.

Milk kept at 3° to −7°

Freshly frozen	200,000 bacteria per c.c.
After 1 day	105,500 bacteria per c.c.
After 2 days	72,300 bacteria per c.c.
After 3 days	62,000 bacteria per c.c.
After 4 days	46,400 bacteria per c.c.
After 7 days	44,000 bacteria per c.c.
After 14 days	40,500 bacteria per c.c.
After 21 days	30,300 bacteria per c.c.
After 35 days	22,500 bacteria per c.c.
After 49 days	14,200 bacteria per c.c.

The table shows plainly that it is impossible to sterilize milk by freezing, but as long as it is frozen it will keep; there in no possibility of any microörganisms decomposing a frozen liquid, for the organisms need water above all. If food substances change in cold storage (and some food products do deteriorate), this must either be due to changes other than microbial or the material was not completely frozen as is probably the case with salted butter.

After bacteria are once frozen, they do not seem to be affected by ny lower temperature. Macfadyen and Rowland found that they lerate very low temperatures remarkably well. Many bacteria ere not killed by a twenty hours' exposure to the temperature of quid hydrogen (−252°). Yeasts are not quite so resistant and the ycelium of most molds is easily destroyed by freezing, while the spores e hardier.

THERMAL DEATH-POINT.—Heating above the maximum tempera- ire is quite harmful to bacteria, and the amount of injury increases ith the temperature. Recent experiments have shown that heat does ot kill bacteria instantaneously, but that we have an orderly process s in the case of death by drying. This can be observed only in a ery narrow range of temperature, however, since the death rate rises ery rapidly with the increase of temperature. 10° increase may make ie death rate ten to one hundred times as great, and death is almost stantaneous. For most practical purposes, it is sufficient to state ie time and temperature neccessary to bring about complete sterili- ation. It has become customary to define, as the thermal death- oint, the lowest temperature at which a culture will be killed in ten inutes. As most bacteriologists will use very nearly the same tech- ic, they will have fairly uniform numbers of cells to start with, nd therefore obtain fairly uniform results.

The thermal death-point does not depend upon the species and ie temperature only. It varies with the age of the culture since der cells are less resistant than younger ones especially if heated in ieir own products. The medium in which the organisms are heated also of great significance. The fact that acid liquids, as fruit juices, e more easily sterilized than neutral meat or vegetables is largely ue to a chemical (poisonous) action of the acids upon the bacteria. ut the greater resistance of tubercle bacteria in the sputum compared ith those suspended in salt solution cannot be so readily ccounted for.

A necessary factor for the prompt destruction of organisms by eat is the presence of moisture. The resistance of dry organisms remarkably higher than that of the same organisms in a liquid cul- ire. The following table shows the death-point of yeast cells and ores in a dry and moist state.

THERMAL DEATH-POINT OF DRY AND MOIST YEAST

Variety of yeast	Cells		Spores	
	Moist	Dry	Moist	Dry
Pale ale yeast	65°	95°–105°	65°–70°	115°–125°
Hofbräu yeast	55°	85°– 90°	65°	115°–120°
Saccharomyces pasteurianus	50°–55°	100°–105°	60°	115°

RESISTANCE OF SPORES.—The organisms most resistant to heat are the spores of certain bacteria. In the chapter on moisture requirements attention has been called to the great resistance of spores to drying. We find the same exceptional resistance to high temperatures. Boiling heat will not kill spores readily. Some bacterial spores can stand the temperature of 100° for several hours. In order to kill spores in one heating the temperature must rise to about 110° for fifteen to thirty minutes; this can be accomplished only by heating under pressure. This is not always advisable for sterilizing food substances. While vegetables are usually sterilized under pressure without losing much of their palatability, other foods like milk are changed materially in taste and appearance. To prevent these changes, discontinuous sterilization is sometimes used. This is based upon the following principle.

If milk or any other medium is heated to 100° for about fifteen minutes, all living cells of bacteria, yeasts and molds will be killed except a few spores of bacteria. After cooling, these spores will germinate under suitable conditions and the vegetative cells thus appearing instead of the resistant spores are easily killed in a second heating. A third heating is necessary in order to kill any vegetative cells which may have developed from spores not yet germinated before the second heating. It is essential to have the time between two heatings long enough to allow the germination of spores, and not too long to permit formation of new spores. It is customary to heat on three successive days for fifteen minutes each time. In this case, sterilization is usually complete, while a forty-five minutes' heating at once is not sufficient to guarantee sterilization. Among the substances that are very easily sterilized are cider and other fruit juices, while milk and soil are the most difficult materials to sterilize.

Dry spores will resist still higher temperatures than moist spores. ome dry spores survive an exposure to 140° or 150° for ten minutes. requires a very high temperature to sterilize glass, cotton, gauze, and struments with dry heat. A discontinuous sterilization of dry mate- al is useless, since the spores will not germinate without moisture, erefore their resistance remains unaltered.

The spores of molds are more resistant than the mycelium, but if oist, they all die at 100°. The dry mold spores can tolerate a some- hat higher temperature, but not as high as the spores of many bacteria. east spores and yeast cells are very much alike in their resistance to at. The table on page 220 shows hardly any difference between their sistance.

CHAPTER III

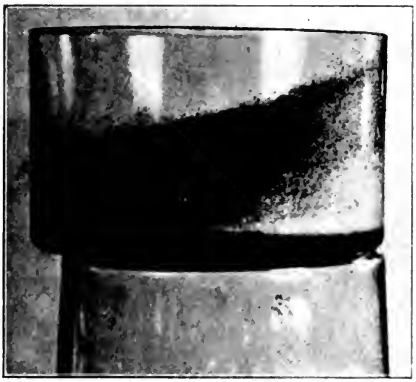
INFLUENCE OF LIGHT AND OTHER RAYS

Microörganisms in their natural environment are temporarily but not usually exposed to light. The organisms of decay, living in soil, in foods, in the intestines of animals, will only occasionally come in contact with the direct rays of the sun. Water bacteria and the organisms on the surface of plants and animals are more commonly exposed to the sun.

FIG. 105.—These plates were heavily inoculated with *B. coli* and *B. prodigiosus* respectively and then were exposed, bottom side up, to the direct rays of the sun for four hours. On the instant of exposure, a figure O cut from black paper was pasted to the plate shading the bacteria underneath. After one, two and three hours the corresponding figures were pasted to the plates. The above picture was taken 24 hours after exposure, proving that three or four hours of direct sunlight weaken and may even kill bacteria. *B. prodigiosus* proved more sensitive than *B. coli*. (*Original.*)

The influence of light varies with its intensity. Direct sunlight has a very harmful effect upon microörganisms. Most bacteria are killed by direct sunlight in a few hours; the time depends upon the organism as well as upon the intensity of light; this again varies with

ıe amount of moisture and dust in the atmosphere, with the time of ıe day and with the season; an absolute measure for the action of light ınnot be fixed, therefore, as easily as with the action of heat in the ther- al death-point. The different colors of the spectrum do not act ike; the part of the spectrum from red to green is practically without fluence upon microörganisms, while the blue light acts strongest ıd the intensity decreases in the violet and ultra-violet. In carrying ı experiments with the influence of light, it must be remembered that ass absorbs ultra-violet rays, and further that the heating of the edium by direct radiation must be avoided (Fig. 105).



G. 106.—Phototropsim of *Rhizopus nigricans*. The mold is grown on gelatin with diffused light coming from right side. (*Original.*)

Yeasts, molds, and bacteria and probably *Protozoa* are equally sensi- ve to light. Even the spores of most bacteria do not show a greater sistance to light, while the mold spores are an exception. The col- ed spores of the *Penicillium*, *Aspergillus* and *Mucor* species can be posed to light for a long time without being killed, but the colorless ores of *Oidium* and *Chalara* show no increased resistance. It is sup- sed that the pigment in mold spores is a protection against light. This not true with the pigment of bacteria. The colored and colorless rains of pigmented bacteria show no difference in their resistance to ght. The only exceptions are the so-called purple bacteria. These culiar organisms, many of which feed on hydrogen sulphide, seem to

thrive better in light than without it. Direct sunlight does not kill them, it rather attracts them and they move toward the light. This is called *phototaxis* or *heliotaxis*. The pigment, bacteriopurpurin, does not take the place of chlorophyl, however, since the bacteria do not produce oxygen in light and always need organic food.

The effect of light upon microörganisms is mainly brought about by a chemical change in the protoplasm, and also, to some extent, by a chemical change in the medium, namely the formation of a peroxide or a similar oxidizing agent.

The germicidal action of light is of importance in the purification of rivers. It is applied also in curing diseases of the skin, as lupus and

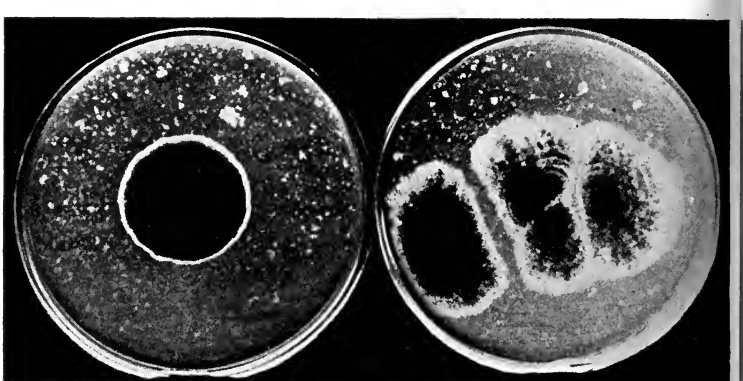


FIG. 107.—Two cultures of an *Aspergillus*, one grown in the dark the other in diffused light, showing rings. (*Original.*)

leprosy, by exposing the diseased parts to a very concentrated light of the electric arc. This light contains plenty of blue and violet rays and is preferable to sunlight because it is always ready for use and its composition and intensity can be controlled easily. Ultra-violet light is used in the sterilization of water and of milk.

Diffuse light is not nearly as harmful to microörganisms as direct sunlight. Long exposures to diffuse light will kill most bacteria, while molds are not at all sensitive. They rather like a very dim light, and many molds grown in a dark room with light only from one side will grow toward the light. This property, which is characteristic for all green plants, is called *heliotropism* or *phototropism* (Fig. 107). It has

len found that molds produce mycelium mostly in the dark, while in ylight sporangia are produced mainly. This difference in the devel- ment during the day and during the night accounts for the concentric ngs which are quite commonly found in older mold colonies, and nich indicate the age of the culture (Fig. 107). Similar rings are casionally found with yeast and bacterial colonies, and are possibly ue to the same influence of light.

X-RAYS.—Of other rays, the invisible X-rays and the radium rays ve attracted the attention of bacteriologists and physiologists. It known that the X-rays will destroy living tissue by long exposures; icroörganisms cannot be considered less resistant. X-rays are used the treatment of microbial diseases of the scalp and skin.

RADIUM RAYS are not so well known, and their bactericidal action is oubtful. The treatment of certain bacterial diseases has been tempted, but it has not been applied as generally as yet as the X-ray ethod. The sterilization of milk and possibly other foods by this ethod has been suggested, but the practical application is at present uite improbable because of the cost and the uncertainty of the results.

CHAPTER IV

INFLUENCE OF ELECTRICITY

The influence of elecrticity upon microörganisms is much less tha one might perhaps expect, if the electriticy as such is considered. direct electric current passing through a nutrient medium will, of cours cause electrolysis which is usually manifested by the formation of ac on the positive pole and of alkali on the negative pole. The acid ar alkali will kill microörganisms, as is discussed in the chapter on chemic influences. In this case, it is not the electricity itself that destroys th bacteria. It is also possible to kill bacterial cultures by passing a alternating current through the medium for some time. No electrolys takes place in this case, still it is not the direct action of the current th acts upon the organisms, but rather the heat produced by the curre passing through a medium of high resistance. If the culture is cool properly the influence of the current is insignificant if at all noticeabl Whenever electricity is applied against microörganisms the effect is co sidered electrochemical.

The electrical current is used in a very small way in the purificatio of sewage. The sewage passes between two iron plates which represe the two poles of a strong current. The electrical sterilization of mi has been patented. Wines are improved by electricity. The steriliz tion of drinking water by ozone is also an application of electricit though of course the ozone once formed by the current acts as a chem ical compound independently of its source, and the same effect wou be produced if the ozone were manufactured chemically.

CHAPTER V

INFLUENCE OF MECHANICAL EFFECTS

PRESSURE.—The resistance of microörganisms to mechanical pressures is very great. Pressures of 3,000 atmospheres* will not kill the majority of bacteria in four hours. They are, however, weakened and some species will die. A specific difference between the molds, yeasts, and bacteria in this particular does not seem to exist. Of the organisms exposed to 2,000 atmospheres for ninety-six hours, *Bact. anthracis*, *Bact. pseudodiphtheriæ*, *M. pyogenes var. aureus*, *Oidium lactis* and *Saccharomyces cerevisiæ* survived, while seven other organisms lost the power of multiplication. Some of these were not dead, however, since they retained their motility for several days. It is noteworthy that high pressure will destroy one quality (multiplication) and not effect another (motility). Pigment-production and virulence of pathogenic bacteria were either diminished or lost completely. The resistance against high pressure is necessary for the organisms which cause the decay of organic matter at the bottom of the oceans. Vertebrates breathe oxygen in the form of gas or have at least an organ filled with gas (fish ladder); the volume of gas is changed considerably by slight changes of pressure; this will affect organisms depending on gas. Microörganisms do not require gas as such. They can absorb gases only in solution. A change of pressure therefore will not cause a change of volume, since liquids have a very small coefficient of compression.

The situation is entirely different if the liquid is not exposed to the pressure directly, but to compressed air. In this case, the chemical effect of the gas is the deciding agent. The higher the pressure, the more gas will be dissolved in the culture medium. The fatal pressure under these conditions will vary as much as the fatal dose of an antiseptic; it depends upon the chemical qualities of the gas, upon the pressure (concentration), upon the temperature, and upon the organism.

*One atmosphere is 1 kg. pressure per square centimeter (or about 15 pounds per square inch).

Some data have been given already in the chapter on oxygen requirements. It was mentioned in that connection that *Bact. butyricum* cannot tolerate more than 0.65 per cent of the total oxygen content in air (0.2 atmosphere); in other words, an oxygen pressure higher than 0.0013 atmosphere will kill the organism. The maximum pressure for *B. prodigiosus* was found to be about 5.4 to 6.3 atmospheres. Very few experiments have been made with other gases. Carbon dioxide at a pressure of 50 atmospheres retards the growth of bacteria in water and will sterilize it in twenty-four hours. Suspensions of pure cultures of *B. typhosus* and *Msp. comma* are killed by 50 atmospheres carbon dioxide pressure in three hours. Milk cannot be sterilized by his pressure but bacteria do not multiply. Carbonated milk has been recommended as a refreshing drink by several investigators. The ordinary market milk will keep about two days longer under the pressure of 10 atmospheres (150 pounds) than without pressure. If pasteurized it is said to keep for a week.

Gravity.—Gravity would have a great influence upon the growth of microörganisms in liquids if their specific gravity were much greater than that of water. This does not seem to be the case however. It has been estimated by accurate weighing to vary between 1.038 and 1.065. Very much higher results (1.3 to 1.5) have been obtained by centrifuging bacteria in salt solutions of varying specific gravity, but these data are not exact since the salt solution will diffuse into the cells and thus increase their weight. The specific gravity being very nearly that of the culture medium, it is plainly seen that gravity has but little influence. The microörganisms will live suspended in the liquid and sediment out very slowly. The slightest current in the liquid will carry them around and distribute them through the medium. The motility is of minor importance; the actual distance covered by motile bacteria has been measured, and under the most careful exclusion of currents in the liquid has been found to be about a millimeter in a minute for *B. subtilis*. This is very slow compared with the speed of the circulating water moved by changes of temperature or other incidental agents.

Yeast cells and other gas producers use the carbon dioxide as a vehicle. The gas bubbling up in the fermenting liquid keeps it constantly in motion and moves the yeast cells against gravity toward the surface where the gas escapes and lets the cells fall back to the bottom.

The production of scums and pellicles on the surface by organisms

hich are heavier than the liquid they float on, is often accomplished by nall gas bubbles between the cells (*Mycodermæ*). In other instances, may be just the floating of cells having oily surfaces.

The growth is influenced by gravity very little. The sporangia of olds are the only exceptions, growing decidedly away from the center f gravity (*negative geotropism*).

AGITATION.—For the majority of microörganisms, the quiet, undisurbed growth of the laboratory culture is the normal or the ideal one. uch cultures, if shaken for a considerable time, show a decrease of livg organisms, and it is possible to sterilize cultures by continued shakg. The effect is not a simple mechanical breaking or tearing of the ells. The bacteria break up into the finest particles. This is also the ase if cultures are exposed for several days to the trembling motion aused by the working of very heavy machines. There is no grinding or earing effect but the cells break to pieces just the same.

A slight and slow agitation seems to be advantageous for many culures, only continuous heavy motion proves harmful. Different organms show wide variations in their resistance to agitation.

DIVISION III

Chemical Influences

CHAPTER I

STIMULATION OF GROWTH

The influence of chemical substances upon microörganisms may be helpful or harmful, or not noticeable. As helpful must be considered above all the food compounds. Unless given in such large doses as to cause a physical or osmotic effect they will stimulate the development. Other substances too, which are not food, can also act as stimulants. It is a recognized fact of long standing that many poisons in very small doses will stimulate. This applies to the most highly developed animals and plants as well as to microörganisms. Raulin noticed in 1869 that *Aspergillus niger* grew very much better in a nutrient solution if a small amount of zinc salt was added. He considered the zinc, therefore, as a necessary constituent of the mold cells. Alcoholic fermentation can be stimulated by metallic salts. It is believed by some physiologists that, as a law of nature, every substance that is injurious in a certain concentration is a stimulant in a lower concentration. A similar action of certain chemical compounds upon enzymes has been noticed, retarding in high concentrations, stimulating in weaker solution.

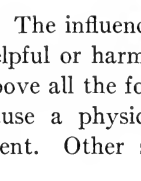


Fig. 108.—Chemotaxis. (*After Fischer.*)

Chemotropism and Chemotaxis.—Microörganisms manifest their preference for certain foods not by a stimulated growth alone. They also make efforts to obtain better food by growing or moving toward it, which is not a manifestation of a rudimentary intellect. Such reactions of microörganisms may be accounted for largely by chemical or osmotic

frces. In a solid medium the hyphæ of molds will grow toward the lst source of food supply. This growth on account of chemical ;mulation is called *chemotropism*, analogous to the *phototropism* growth toward light. If some injurious compound is offered, le hyphæ will grow away from it. Thus we have to distinguish tween *positive* and *negative chemotropism*. The motile organisms, cteria as well as protozoa, demonstrate their preference for certain od compounds by swimming toward them. This is called *chemotaxis* ig. 108). Here also a *positive* and *negative chemotaxis* must be stinguished, the latter taking place if injurious substances are present.

CHAPTER II

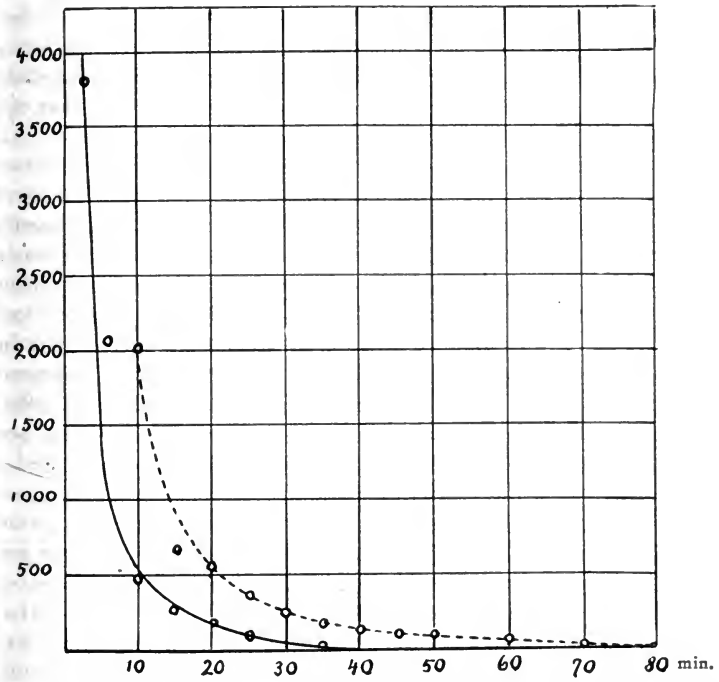
INHIBITION OF GROWTH

Poisons, Germicides, Disinfectants, Antiseptics, Preservatives.—A great number of inorganic and organic bodies will destroy life in comparatively weak solutions. These substances are called *poisons* if they are considered in their effect upon man and animals. In their application to microörganisms they are generally called *germicides* (germ-killers), or *disinfectants* if the emphasis is laid upon the prevention of infection rather than upon the actual killing of the microörganisms. Analogous to the general term germicides, the terms *bactericide* and *fungicide* are used occasionally. The term *antiseptic* means a prevention of sepsis which may be accomplished by checking the growth without necessarily killing all microörganisms. The meaning of the word *preservative* is practically the same, only the latter is used more commonly in relation to foods, feeding stuffs and preparations of similar origin, while the word *antiseptic* is largely used in relation to microbial diseases. A strict line cannot be drawn between any of these definitions. A disinfectant, if diluted, becomes an antiseptic. A strong salt solution is an antiseptic for some organisms and a disinfectant for others. Of the above expressions, germicide is the most definite, but is not so commonly used as the others.

Mode of Action.—The action of a poison upon the cell is generally considered an action upon the protoplasm. The poison is supposed to combine chemically with the cell plasma producing compounds which interfere with the continuation of the life processes and thus cause death. If the cell has been subjected to the action of the poison only a short time, it can be saved by removing the poison. Bacteria can be treated with mercuric chloride ($HgCl_2$) so that they will no longer develop if transferred to a fresh medium. If the mercuric chloride is removed from the cell by means of hydrogen sulphide, some of the organisms may be revived.

The mode of death through poison is the same as that through

eat or drying. The number of cells dying in a given time interval is roportional to the number of cells surviving. In the last five years, his has been tested and found true with practically all disinfectants. ig. 109 shows the curves plotted from data obtained with *Bact. anthracis*, he full-drawn line representing the number of live spores in .21 per



IG. 109.—Curve of disinfection. Spores of *Bact. anthracis* in mercuric chloride solution. (*After Chick.*)

ent of mercuric bichloride, the dotted line the same in .11 per cent olution.

The (apparent) resistance of the few remaining cells is of great im- ortance in those applications of disinfection where a thorough kill- ng of all bacteria is intended, *e.g.*, in the treatment of drinking water. Our ideas of the efficiency of a disinfectant would depend, therefore,

upon the accuracy with which we can prove the presence of a certain bacterium.

Factors Influencing Disinfection.—The efficiency of a disinfectant depends upon several factors. Moisture is necessary—a dry poison has only a very slow action upon microörganisms. For this reason, absolute alcohol has not nearly the same germicidal power upon dry bacteria as diluted alcohol; the strongest poisonous effect is obtained by a 50 to 70 per cent solution. The necessity of moisture is further demonstrated in the sterilization with gases, as with formaldehyde. The effect of formaldehyde gas without the provision of a very moist atmosphere is surprisingly weak.

The temperature is also quite an important factor in the study of disinfectants. Since poisoning is supposed to be a chemical effect, it must be expected that the poisoning process like other chemical processes will take place faster at a higher temperature. As a matter of fact, the death rate through poisoning is usually doubled or trebled by a temperature increase of 10°. Above the optimum temperature, where the growth is not very vigorous, and when the disinfecting power of the poison is increased considerably by the higher temperature, a very small amount of poison will have a very strong germicidal effect. The combination of high temperatures with a disinfectant has been suggested as a means of sterilizing foods. This has been tried in the case of milk with hydrogen peroxide at 50° to 60°.

It makes a considerable difference whether the organisms which are tested with a certain disinfectant are in a culture with their food material, or suspended in water or salt solution without any food. It is very probable that part of the disinfectant is acted upon by the food products which are partly protein substances and are in many ways similar to the protoplasm of the bacterial cells. It is especially difficult to poison bacteria in blood, pus, or similar material. The sensibility of the microörganisms in pure water is remarkable. Very small doses which would not be considered efficient under any other condition, will destroy microörganisms in pure water. The concentration of chloride of lime which is sufficient to sterilize drinking water, does not at all suppress the development of bacteria in sewage.

The influence of the number of cells is evident from the above explanations of the mode of action, and from the curves of disinfection. The concentration of the poison is of course of greatest importance.

Recent investigations have shown the rather unexpected fact that the efficiency of a poison is not proportional to its concentration. If a certain poisonous solution is diluted with an equal volume of water, we might expect it to be half as poisonous as before, but depending upon the chemical nature of the poison, it may be more poisonous than expected, or considerably less. It follows from this that two different poisons of the same intensity, if diluted in the same proportion, may not have the same intensity any more.

Microörganisms will gradually become accustomed to certain poisons, and become more resistant. This principle has been utilized in the manufacture of distilled alcohol; yeasts have been cultivated which can tolerate a high concentration of acid; the acid serves to suppress bacteria producing undesirable fermentations.

The age of the culture and the stage of development will naturally change the resistance of a species materially. The old cultures which are past the culmination of growth will be much more sensitive to any poison unless a spore-producing organism is under test. In this case, we find a greatly increased resistance, similar to the increased resistance of spores against drying and heat.

THE CLASSIFICATION OF DISINFECTANTS is very difficult as long as we cannot explain completely the process of poisoning. It is impossible to arrange them according to the intensity of action, because the intensity of influence depends not only upon the disinfectant, but also upon the species of organisms. Some yeasts can resist ten times as much alcohol as certain bacteria. Formaldehyde is not nearly as strong an agent with molds as it is with bacteria. The disinfectant concentration of a poisonous substance is not absolute. The simplest method of grouping is by chemical structure and qualities. Of the following natural groups can be distinguished acids (inorganic and organic), metallic salts, hydrocarbons (aliphatic and cyclic), alcohols (aliphatic and cyclic), aldehydes, anæsthetics, essential oils, oxidizing agents and reducing agents.

The first three groups, acids, alkalies and salts, are distinguished from the rest as electrolytes; the strength of acids and alkalies (chemically speaking) is measured by the degree of electrolytic dissociation. The disinfectant value follows largely the same law. The strongest acids in the chemical sense are also the strongest disinfectants. There are exceptions, however, where, besides the poisonous effect due to

the degree of dissociation, there is a specific effect due to the chemical structure, as is the case of nitrous, salicylic and hydrocyanic acids. The same is true of alkalies. With metallic salts, the action will depend mainly upon the metal in solution, but the electrolytic dissociation is also of importance. NaCl will decrease the dissociation of mercuric chloride ($HgCl_2$) and decrease also its disinfectant power. Mercuric chloride dissolved in absolute alcohol is not dissociated. In this case, it has almost no action upon bacteria.

Acids are not commonly used as disinfectants, except in the household, but they play a certain rôle in nature. The common fruits contain so much acid that bacteria cannot easily attack them; the decaying of fruit is almost exclusively due to molds which have a preference for acid media. The acid in the stomach of man and animals plays an important rôle as a sterilizing agent for the food. Many microörganisms are killed in the stomach. In the household, the natural acidity of fruit helps in keeping canned fruit, preserves and jellies. Especially in heating, the acid together with the high temperature has a very strong germicidal effect. Vinegar is often used to preserve fruit and vegetables; in some parts of the country, meat is kept in buttermilk. Benzoic and salicylic acids are often used in the preservation of fruit and vegetables. Their poisonous influence is not so much due to the acid reaction but to the specific chemical character of these compounds.

Of the alkalies, only one is used extensively, namely, lime; quicklime (CaO) is considered a valuable disinfectant for excreta in privy vaults; it is universally applied as a whitewash in stables, barns, poultry houses and similar buildings. Quite commonly, it is used as "milk of lime" (one part of slaked lime with four parts of water). It should be kept in mind that the calcium oxide unites with the carbon dioxide of the air and thus gradually loses its disinfecting power.

Of the metallic salts, many are well-known germicides. The most powerful disinfectant is mercuric chloride ($HgCl_2$) which is one of the standard disinfectants. It is generally used in a dilution 1 : 1000 which is sufficient to kill all vegetative cells as well as spores in a few minutes. Quite commonly, hydrochloric acid or salt is added, to prevent coagulation or precipitation of slimy or albuminous matter which would protect the enclosed bacteria from immediate contact with the poison. The addition of hydrochloric acid or any chloride

decreases somewhat the disinfectant value for bacteria suspended in distilled water because it decreases the electrolytic dissociation.

Another disinfectant of remarkable strength is silver nitrate; it is not used commonly because of its high price. It also decomposes easily and leaves dark spots on the skin and clothes. Of the other metallic salts, copper and iron sulphate are not used extensively, though recommended for the disinfection of feces. Zinc sulphate may be applied to mucous membrane the same as silver nitrate. Many other salts may be used occasionally for disinfecting purposes, though the expense or undesirable qualities prevent their common application.

The alcohols are well known for their poisonous effects, but the value of ethyl alcohol as a disinfectant is usually overestimated. It takes quite strong alcoholic solutions, more than 20 per cent, to kill certain yeasts and the spores of some bacteria in less than a day, and a complete sterilization by alcohol in a few minutes cannot always be guaranteed even with 50 to 60 per cent solution. It has already been mentioned that desiccated organisms are very resistant to concentrated alcohol, more so than to a 50 per cent mixture. Methyl alcohol is weaker, the higher alcohols, especially amyl alcohol, are stronger disinfectants than ethyl alcohol. They all give good results in the presence of water while the absolute alcohols have scarcely any effect upon desiccated bacteria. None of these alcohols in whatever concentration they may be used, can be relied upon to kill bacterial spores.

Stronger germicidal effects can be obtained by the alcohols of the benzol group, of which phenol or so-called carbolic acid (C_6H_5OH) is the simplest representative. Phenol, like ethyl alcohol, is not as effective as is commonly believed. It is applied in solutions from .5 per cent to 5 per cent ordinarily, but it usually takes a long time even for the 5 per cent solution to kill vegetative cells as *Bact. tuberculosis* or *B. coli;* it is inefficient against anthrax spores. More powerful are the higher cyclic alcohols, of which the cresols are examples. They are used extensively as disinfectants and antiseptics. They are, together with phenol, coal-tar constituents and are sold commercially under many different names, either pure or mixed with soap or other disinfectants which make them emulsify readily in water. The cresols are almost insoluble in water, and not as effective in solutions as they are in

emulsions. The disinfecting properties of tar come from the cresol contained in it.

Hydrocarbons are used only for laboratory experiments as very weak antiseptics. The aliphatic bodies, as methane, etc., which constitute a large part of coal gas, have very little if any effect upon bacteria; gas is used occasionally in place of hydrogen for growing anaerobic bacteria. Benzol, xylol, and toluol are antiseptics, if shaken frequently with the liquid to be protected, but they are not reliable as disinfectants. The same is true with the comon anæsthetics, ether and chloroform. The high prices of these agents forbid their general use, but they are sometimes used for laboratory work.

The essential oils have a little more practical importance. Some of these are the main constituents of mouth washes, especially the oil of peppermint (menthol), of thyme (thymol), and of eucalyptus (eucalyptol). Their action is very weak, however. The volatile oils of spices have to be considered in the preserving of fruit, pickles, catsups, and other food products. Though the antiseptic value in general is insignificant, certain microörganisms are sensitive to certain spices. The bacteria of the mesentericus group are said to be suppressed entirely by quite small quantities of garlic, while others, like the lactic bacteria, are not affected at all. Cloves, cinnamon and alspice are the most efficient spices, while the disinfectant powder of black and white pepper and mustard is very small.

The most important disinfectant has not been mentioned, because it does not belong to any of the above groups. This is formaldehyde. Formaldehyde (HCOH) is a gas, soluble in water to the amount of 40 per cent at room temperature; it does not attack metal, clothing, woodwork, and is, therefore, preferable to many other disinfectants for sterilizing rooms. It kills spores of bacteria in a short time in a 1 : 1000 dilution. Its greatest importance lies, however, in its gaseous nature, because it can be applied to rooms and buildings by simply evaporating it. The saturated 40 per cent solution can be evaporated directly or by generating steam which passes through the formaldehyde solution; this latter method has the advantage of saturating the air with moisture, which increases the power of the formaldehyde gas. Formaldehyde can also be obtained in a dry form; it polymerizes to a white crystalline substance, paraformaldehyde ($(HCOH)_3$) which can be changed back to formaldehyde gas by gentle heating. This paraformaldehyde is com-

monly used instead of the liquid, because it is more easily handled and is quite inoffensive in its solid form, while the formaldehyde solution has a very penetrating odor and is exceedingly harmful to the mucous membrane of the respiratory organs.

Of the oxidizing agents, oxygen itself has already been mentioned. Though it is able to destroy certain anaerobic bacteria, it cannot be called a disinfectant. For this purpose, oxygen must be activated; such oxygen can be obtained in the form of ozone (O_3). It is formed in air under the influence of electric discharges and can be produced at a price low enough to allow its application for use in the sterilization of water. It has also been recommended for preservation of milk.

Hydrogen peroxide (H_2O_2) resembles ozone in its chemical reactions; it changes readily to $H_2O + O$, and this oxygen atom in the nascent state is quite effective as an oxidizing agent. For an antiseptic, it must be used in at least a 1 per cent solution, and for an absolutely reliable disinfectant a still higher concentration is required. It loses its disinfecting property easily because it is decomposed readily by the peroxidases of tissues and organic liquids as blood, milk, and pus. It is used in the preservation of milk. Hydrogen peroxide is slowly decomposed by the katalase of milk thus disappearing completely.

Chlorine in its gaseous form is not used as a disinfectant, though its germicidal power is quite strong. The so-called "chloride of lime," manufactured by absorbing chlorine in slaked lime, gives in water hypochlorite and free chlorine; these substances are good germicides and chloride of lime is used in the disinfectant of privy vaults, and other places in which it may be employed without injury. Hypochlorite is now used with great success for rendering safe drinking water and sewage; it has also become the basis of some commercial disinfectants.

Potassium permanganate is only incidentally used as a disinfectant. Its chemical qualities prevent an ordinary use.

Sulphurous acid, or sulphur dioxide (SO_2) was for a long time a standard disinfectant and is still used occasionally for fumigating rooms, stables, barns and out-buildings though it is substituted more and more by formaldehyde which can be applied almost as easily. The burning of sulphur is an extremely simple process, but it requires a moist air to disinfect properly, and under these circumstances it will attack metal, dyes of clothing and even the fiber itself.

In addition to these disinfectants which are used outside of the human body, or applied to its surface only, there have come into use during recent years, several disinfectants which are injected into the body to kill the microörganisms in the blood. Among these might be mentioned the colloidal metals, mainly colloidal silver which is sold under various trade names, *e.g.*, collargol. It is given especially in pneumonia, but its action upon the bacteria directly is very insignificant, though it greatly stimulates phagocytosis. Further, there is to be mentioned ethoxyl, given against the protozoön of sleeping sickness, and the latest and most discussed of all, salvarsan, an organic arsene compound, against syphilis.

DIVISION IV

Mutual Influences

INTRODUCTION.

The biological relations of microörganisms are of the greatest importance in nature. Pure cultures in nature are very rare and of exceptional occurrence; they are hardly ever found except in certain diseases of man, animals and plants. Generally, nature works with mixed cultures. All natural fermentations, decompositions and putrefractions are accomplished by a number of different species among which perhaps one dominates, but is influenced by the rest. The study of the mutual relations of microörganisms is in the very first stage as yet; practically all laboratory work is done with pure cultures. The experiences obtained with pure cultures are not sufficient to explain all microbial activity in nature.

There are many possibilities of mutual influence between different organisms. Generally three main cases are distinguished: *symbiosis*, where two organisms profit by the combination; *metabiosis*, where one profits by the other's action without benefiting the other in return, and *antibiosis*, where one organism injures the other. These cases cannot be separated strictly. The relations are not always constant through the entire development of the cultures; an originally beneficial influence may change to an injurious one in a few days. Many terms have been coined to designate all these various possibilities, but in order to avoid this multiplicity of more or less indefinite names for the various relations, the general term "association" has come into use, especially when the relationship is not well understood.

SYMBIOSIS.

Symbiosis is not very common among microörganisms, and it is difficult to find examples where true symbiosis exists through the entire

development of both organisms. The association of lactic bacteria and *Oidium lactis* in milk is, for a certain period at least, a symbiosis. The bacterium will produce only a certain amount of acid, and then it can grow no more because the acid is too strong; the mold will destroy the acid and thus gives the bacterium a chance for continued activity. The bacterium produces the acid which the mold likes; the mold in turn removes the excess acid which otherwise would check the bacteria activity.

True symbiosis is more common in the relation of microörganisms with higher plants and animals. The standard example in the plant kingdom is *Ps. radicicola* in the nodules of legumes, feeding on carbohydrates provided by the plant and furnishing the plant nitrogen from the air which the plant cannot assimilate directly. The typical example in the animal kingdom is *B. coli* in the intestine of animals, being nourished by the food of the animal and rendering the food more easily digestible.

METABIOSIS

Metabiosis may be considered a one-sided symbiosis; two organisms live together, but only one is benefited, the other remains uninfluenced or later may be injured by the association; the latter case is the most common. In this relation, one usually prepares the food for the other. It has previously been mentioned that the metabolic products of one species serve as food for another species, thus breaking up the various organic compounds step by step to smaller and simpler molecules. Quite commonly, each step is accomplished by a different species of microörganism. Consequently, metabiosis is a very common occurrence among microörganisms.

The classical example is the two nitrifying bacteria: the nitrate bacterium is unable to oxidize ammonia, and depends entirely upon the nitrite bacterium to oxidize the ammonia to nitrite; then, and only then, can the nitrite bacterium grow.

The relation between yeasts and acetic bacteria is also very well known. The yeast ferments the sugar to alcohol, and then the acetic organisms oxidize the alcohol to acetic acid. The yeast is in no way helped by the acetic bacteria, while these could not form acetic acid from sugar readily. These bacteria depend upon the action of the alcohol-forming yeast. Other cases of metabiosis are found in the

association of lactic bacteria with certain protein destroying organisms. The lactic bacteria often develop much better if the protein bacteria grow together with them or have grown previously in milk. Metabiosis does not require the growth of the two associated organisms at the same time. The effect will be the same if first the one and later the other develops, and even after the first organism is killed or removed, its effect upon the pure culture of the second will still be noticed. This does not occur in the case of symbiosis.

One species can favor the development of another by other means than food provision or preparation. Certain bacteria cannot live in acid media, and molds or mycodermas destroying the acid will render possible the growth of these bacteria though they do not provide them with food. This is the case in the ripening of certain soft cheeses. Another example is the production of heat by fermenting organisms in manure, hay, ensilage, enabling the development of thermophile organisms. A very interesting and important problem is the growth of strictly anaerobic bacteria near the surface of liquids in association with some aerobic bacteria. How this is really possible cannot be satisfactorily explained. Though the aerobic bacteria continuously remove the oxygen from the water a certain amount will remain, sufficient to prevent the growth of the anaerobic bacteria under ordinary conditions. There seems to be a certain protective influence derived from the aerobic bacteria, the nature of which is unknown.

ANTIBIOSIS

The standard examples of antibiosis are the alcohol production by yeast in sugar solutions and the acid production by lactic bacteria in milk. Fresh cider contains a large number of bacteria, yeasts and molds; some of these organisms cannot develop in the acid medium, but many will begin to grow. Some of the bacteria will produce or destroy acid, others may begin to work on the nitrogenous material of the cider, and the yeasts produce alcohol and carbon dioxide. The carbon dioxide will soon saturate the cider and begin to bubble up, thus removing the other gases. The molds will stop growing if the oxygen is taken away, but some of the bacteria may continue growing until the alcohol concentration checks their further development. They first cease to grow, then cease to produce acid and finally die, while the yeast is still continuing in the fermentation.

In the lactic fermentation of milk, *Bact. lactis acidi* combats all other organisms by a rapid production of lactic acid. Though it is present in fresh milk only in very small numbers, its rapid growth and the formation of acid which will check and even kill most other bacteria soon makes it the dominant organism in the flora of milk, and at the time of curdling, it is often difficult to find any other organisms besides the lactic bacteria. In the preceding chapter was mentioned the metabiosis of certain protein-digesting bacteria with *Bact. lactis acidi*. This metabiosis can be considered as such only from the standpoint of the lactic organism. The protein bacteria are killed by the acid formed by the rapidly growing lactic bacteria. From the viewpoint of the protein bacteria, the relation is antibiosis. Another illustration of antibiosis is the acetic fermentation. The formation of acetic acid prevents the development of all bacteria and of most yeasts and molds.

In all these cases, the deciding agent is a well-known chemical compound. In other combinations, the principle is unknown. *Bact. lactis acidi* will check the growth of *B. subtilis* not only in milk where it forms acid, but also in sugar-free broth where acid production is impossible. Acetic bacteria act upon the yeast cells not only by means of the acetic acid produced, but also by some other, unknown agent, since vinegar is more injurious than the corresponding amount of pure acetic acid in water. A very remarkable organism is *Ps. pyocyanea;* it secretes a substance, *pyocyanase*, which will kill and dissolve the cells of other bacteria rapidly.

Parasitism, which would be classified under antibiosis, has not been found to exist among bacteria or yeasts; but we know of cases where one mold grows on the other; this is especially true with the largest representatives of the mucor family, which are often attacked and sometimes killed by smaller fungi.

RELATIONS BETWEEN CELLS OF THE SAME SPECIES

That cells of the same species will also influence each other, may well be assumed. The simplest relation will be the competition for food. This will be the case in nature more commonly than in laboratory media which are, as a rule, so rich in nutrients that development ceases before all food is used up.

The cause for cessation of growth in a culture is of great theoretical and practical interest. Apparently there are various factors concerned in this. Lack of food, or of one single essential food compound, may be the cause. This is found sometimes in media where it would be least expected. Some strains of *Strep. lacticus* are supposedly limited in milk by the lack of available nitrogen; they cannot attack casein readily and albumin; besides these proteins, nitrogen compounds are not plentiful. Addition of peptone increased the maximum number of cells from 0.7 billion to 2.5 billions per c.c. More commonly, however, growth is checked by the accumulation of metabolic products. Yeasts are checked by the alcohol, and acid-formers by the acid, urea bacteria by the alkali. In many of these cases, the removal, or neutralization, of the inhibiting product will bring about new development.

The harmful products accumulating are not always of such simple nature. Some very interesting observations have been made during the last ten years. Eijkmann, as the first, found that *B. coli* reached its maximum growth in gelatin at 37° in a few days, and that this gelatin, after hardening at 20°, would not support growth after streaking with a young culture of the same organism; but after this gelatin had been heated at 60° for half an hour, *B. coli* grew on it as well as on fresh gelatin. Broth in which *B. coli* had grown became fit again for growth of the same bacillus after filtration through porcelain. The inhibition of growth is, in this case, due to a compound which resembles a toxin in many respects. The importance of such investigations to general physiology is evident.

PART III

APPLIED MICROBIOLOGY

DIVISION I*

MICROBIOLOGY OF AIR

CHAPTER I

THE MICROORGANISMS OF THE AIR AND THEIR DISTRIBUTION

The atmosphere is not the normal habitat of bacteria, for growth and multiplication cannot take place in it under ordinary conditions. The phrase "microörganisms of the air" is therefore somewhat ambiguous. The small size of microörganisms enables them to remain suspended for considerable periods when physical forces have separated them from the substrata on which they have developed.

MICROÖRGANISMS PRESENT IN THE AIR.—Molds, bacteria, and yeasts are all found in the air under certain conditions. The first two are usually relatively abundant, the latter are less common.

The common molds have adapted themselves for the most part to wind distribution. They bear spores that are small in size and possess a surface that is not readily moistened. These spores are resistant to desiccation and light and remain viable for a considerable tine even under unfavorable conditions. Furthermore, the fruiting bodies of many, though not all molds, show a distinct negative hydrotropism, *i.e.*, the mycelium remains in contact with the moist substratum while the threads which bear the spores rise at right angles to it. These latter are so sensitive that they can detect slight differences in the moisture content of the air and grow in the direction which will bring the spores into

*Prepared by R. E. Buchanan.

the driest situations. A slight current of air will detach the spores from these structures and carry them long distances.

Bacteria and yeasts lack the specific adaptations for wind distribution found in molds. The material upon which they have been growing must be dried and pulverized before they can be blown about. Many species produce spores or other resistant cells, and physiologically are as well adapted for air distribution as are the molds.

Occurrence in the Air.—Microörganisms are found free in the air, attached to particles of dust, or enclosed in minute drops of water. Mold spores are commonly free or in unattached clusters. Bacteria and yeasts are usually associated with dust particles, frequently the pulverized substratum on which they have been growing. Not all dust particles have living organisms attached. It has been computed that in the air of London during a fog there is only one living organism for over thirty-eight millions of dust particles. Microörganisms are sometimes sprayed into the air with water. Droplets containing bacteria are thrown off in the saliva in coughing or in speaking, and from the surface of fermenting liquids on which bubbles are bursting. When the drop is small enough, the air currents keep it in suspension and the water soon evaporates and frees the organism. This brings about the condition first discussed, free bacteria in the air. The decrease in weight and size incident to this loss of water probably accounts for the fact that the so-called "infectious droplets" are sometimes carried for considerable distances.

How Microörganisms Enter the Air.—In comparatively few instances do microörganisms possess mechanical devices for projecting the spores or other cells into the air for wind distribution. Usually the organism is passive and is freed only by air currents or by mechanical agitation. Some molds, as has been stated, release their spores even in the presence of moisture, so that complete desiccation is unnecessary for their dispersal. Bacteria and yeasts, on the other hand, are not usually given off from moist surfaces. Only when dry and pulverized can the bacterial medium be readily blown about. Hansen found that in the immediate vicinity of a heap of decaying malt, the air was comparatively free from bacteria. Winslow has shown that sewer air is frequently practically free from bacteria although the surface with which it comes in contact teems with bacterial life. Mechanical agitation often throws large numbers of organisms into the air. Moving hay and straw

rooming animals, sweeping a floor or carpet will multiply the dust and acterial content of the air many times. In a similar manner, tiny, erm-holding droplets may be scattered by the splashing of sewage or of rmenting or putrefying liquids, and in speaking, sneezing or coughing.

Conditions for Subsidence of Bacteria.—The length of time uring which an organism may remain suspended in the air is dependent pon several factors. Small particles settle out more slowly than large r the reason that as the size of an object is decreased, the surface area ecreases less rapidly proportionately than the volume. The lifting ffect of air currents depends upon the ratio of surface area to volume nd specific gravity. The smaller the object, therefore, the greater is he resistance to subsidence. Consequently, bacteria usually settle ut of air very slowly if free in a quiet atmosphere. The time of sus- ension is determined also by the velocity of the air currents. While onsiderable velocity may be necessary to dislodge microörganisms and ring them into suspension, a very slight air current will sustain hem. Winslow has found that a current of 17 inches per minute is ufficient to sustain *B. prodigiosus*. The relative humidity of the air is lso an important factor. In a supersaturated air solid particles, such s bacteria, become foci of condensation for water and quickly settle ut. When dust is present in considerable quantities, and certain elec- rical or moisture conditions exist, flocculation occurs and the larger odies so formed subside rapidly. The character and abundance of urfaces with which the suspended particles may come in contact also lay an important part. Moist surfaces are much more effective in etaining particles than those which are dry.

Determination of the Number of Bacteria in the Air.—The umber of bacteria in the air is frequently determined by exposing open etri dishes of gelatin or agar in different places for definite periods. This is a comparative quantitative method only. The number of colo- ies developing upon these plates will give the number of dust particles aving living spores or cells upon them that fall in the given area under he conditions of the experiment. Evidently this is of value only for ough comparative work as constantly shifting currents of air usually ntroduce great errors. A somewhat more accurate method is to draw neasured volumes of air into a flask, the bottom of which is covered with a layer of gelatin or agar. The colonies which develop represent he number of organisms which settle out from the given volume. More

accurate results still may be obtained by drawing measured volumes of air in small bubbles through liquid gelatin. Practically all of the particles will be retained and the number of colonies which develop may be counted. This method is sometimes modified by drawing the air through a definite volume of water, care being taken to insure sufficient contact of air and water to remove all dust particles. A proportionate part of the water is then plated and the number of organisms estimated. Air is sometimes drawn through a filter made of sugar, sodium sulphate, or sodium chloride, and this material then dissolved in water and plated. Sand, asbestos, glass, etc., are sometimes used as air filters, then thoroughly washed, and the wash water plated.

Relative quantitative examination of the air is of more historical than practical importance. It has been useful in the development of the germ theories of fermentation and of disease and in overthrowing the theory of spontaneous generation. There is so little ordinarily to be learned by a study of the air flora that a comparison of plates exposed directly will usually suffice. Where more accurate results are desired, one must resort to one of the filtration methods discussed above.

Qualitative determinations of the species of air organisms are not often made. When necessary it may be done by simple examination of the colonies developed on the plates or by animal inoculations made from the water used in the air filter. It is sometimes necessary to vary the composition of the medium used in order to favor the development of certain types of organisms desired, for example, a higher precentage of molds will be found and a more luxuriant development will take place if wort agar or acid gelatin is used.

Number of Bacteria in the Air.—The number of bacteria in the air is determined by a variety of conditions. The velocity of air currents and the nature of the surface with which these currents will come into contact, are probably most important. Bacteria are usually more abundant on quiet days in the air of buildings than out of doors, but on windy days the reverse is true. They are often more abundant in cities than in the country. Fewer are found at high altitudes and over large bodies of water. Frankland found that there are fewer in winter than in summer. They are washed from the air during rains. Bright sunlight destroys many. The nature of the soil and the vegetation covering it has a marked influence. The following figures from various

thors are appended to serve as an index to what may be expected in te air content of bacteria.

Locality	Number of organisms per cubic meter	Observer
tdoor air, Boston	100–150 bacteria. 50– 75 molds.	Sedgwick and Tucker.
en air	100–150 bacteria.	Fischer.
en field	250	Uffelman.
acoast	100	Uffelman.
untain altitude, 200 meters	0	Pasteur.
nt Blanc	4– 11	Ellis.
itzbergen (Arctic Regions)	0	Levin.
ddle of Paris	4,000	Ellis.
ris Street	3,500	Fischer.
ilor's Room in Whitechapel	17,000	Ellis.
ot Workshop	25,000	Ellis.

Species of Organisms in the Air.—*Penicillium* is the most com- on mold isolated from the air. Next in importance are *Mucor*, *hizopus*, and *Aspergillus* in the order given. In addition to these a nsiderable number of species of hyphomycetous molds are occasion- y found. *Torulæ*, but not true yeasts, are usually common. Bac- ria are either spore-bearing soil bacilli or cocci. Of the former, *B. sub- is*, *B. mycoides*, and related forms are ubiquitous. *Sarcina lutea* and *rcina aurantiaca* and certain other chromogenic cocci are to be found almost every plate exposed. Since the air does not have a true flora, e species as well as the number of bacteria present must depend en- ely upon the character of the environment.

CHAPTER II

MICROBIAL AIR INFLUENCE IN FERMENTATION, DISEASES, ETC.

AIR AS A CARRIER OF CONTAGION.—There are many popular mi conceptions of the influence of air upon health. Experience ear taught that exposure to the night air in certain localities or to swan air during certain seasons was generally followed by disease. Natu ally, the air itself was held responsible. We know now that certa fevers, malaria, etc., are caused in every instance by infection wi specific microörganisms and that these organisms are not usually ca ried by the air but by insects, such as the mosquito, in water and foo Nor can the emanations from decaying organic matter or sewer gas its be held to produce disease directly. Before the establishment of t germ theory of disease, leading sanitarians held that sickness w induced by the gases from the decaying organic matter, by the effluv from cesspools and by sewer gas. However important the places nam may be in harboring disease microörganisms, we have learned that t air itself rarely acts as a carrier. Sewer gas has been shown to be u usually free from bacteria. Hazen says, "After many years of exp ience and long-continued investigation, there is not the slightest reas to believe that infectious diseases are carried by the air of sewers."

Undoubtedly the air does play some part in the carrying of dise germs. In certain diseases, as the exanthemata (smallpox, meas etc.), the infecting agent may be present on the dry skin and may blown about and inhaled. This means, however, is not establish In certain nasal, tracheal, and pulmonary infections, the organis may be spread through speaking, sneezing, and coughing, for the in tious droplets, as has been seen, remain suspended for a time in air. Pyogenic cocci are present in the mouth and care must be use surgical operations that the mouth is so protected that none of th organisms gain entrance to wounds. Rarely, if ever, are intest infections, as typhoid or cholera, spread through the air. We may th

fore conclude that air is of secondary importance as a carrier of infection. It may be of importance in a crowded workroom, but even under these conditions it is probable that transmission of infection comes about more frequently through actual contact or through food and drink.

Organisms of the Air and Fermentations.—A uniform inoculation with soil bacteria such as produce the nodules on the roots of leguminous is obtained over considerable areas through the action of the wind in blowing dust particles. The bacterial flora of milk is to some extent dependent upon air currents as is also the development of the molds necessary to the proper ripening of cheese, such as the Camembert. Acetic, butyric, and other organisms are likewise distributed in this manner. The organisms responsible for putrefaction and decay, the molding and spoiling of foods are wind-borne.

Freeing Air from Bacteria.—Air is most commonly freed from bacteria by sedimentation, for this is the ultimate fate of most dust particles. We have seen that they gradually subside in a quiet atmosphere. When large quantities of pure air are required, dust and bacteria may be removed by passage through a spray of water or through various types of filters, such as cotton, glass, wool, etc. A familiar example of this type of filtration is the laboratory use of cotton plugs in test-tubes. It is sometimes necessary to resort to fumigation to destroy the organisms of the air when an undesirable species is present.

DIVISION II

MICROBIOLOGY OF WATER AND SEWAGE

CHAPTER I*

MICROORGANISMS IN WATER†

Water is necessary in the life of man. Besides its use as a beverage for cooking, and all domestic purposes, it is largely used in many manufacturing industries; therefore, the study of its chemical and biological content is one of the most important features of modern hygiene. All natural waters contain microörganisms, which gain entrance from many sources.

Under the influence of the sun, sea water evaporates and forms water vapor, which we call clouds; and these, driven by the wind over the land, are precipitated as rain and in the form of snow or hail.

Most of this water collects from vast areas into brooks, creeks, rivers, lakes, or in subterranean streams, and finally reaches the sea whence it came.

The water vapor arising from the sea or land contains no organisms but as soon as the vapor is precipitated microörganisms find their way into it. These come from the air and from the soil. Some of them find in water sufficient nutriment for their life and growth; and, because of their constant presence and evident ability to thrive in water, they are sometimes spoken of as belonging to the "*water flora.*" Others, such as

* Prepared by F. C. Harrison.

† For specific details regarding methods of analysis and a fuller presentation of the subject readers may consult any of the following excellent books:

1. Savage, W. G.: The Bacteriological Examination of Water Supplies, London, H. Lewis, 1906.
2. Horrocks, W. H.: An Introduction to the Bacteriological Examination of Water, Lond J. and H. Churchill, 1901.
3. Prescott and Winslow: Elements of Water Bacteriology, 2d Ed., New York, Wiley Sons, 1913.

e soil bacteria, are found only at certain seasons, as after rain or dur-g flood-time, and flourish only for a time; while some few, such as testinal organisms that find their way into water, survive for only short period.

Classes of Bacteria Found in Water

The bacteria found in water are here roughly divided into: (a) natu-l water bacteria; (*b*) soil bacteria from surface washings; (*c*) intes-nal bacteria, usually of sewage origin. But there is no strict divid-g line between these three groups; for some organisms belonging to e water flora are found in the soil, and *vice versa*. Water draining om manured land frequently contains intestinal organisms. The vision, however, is sufficient for all practical puropses.

Natural Water Bacteria.—The natural water bacteria are gen-ally regarded as harmless to man. These organisms are frequently merous in river, lake, and all surface waters; certain species predomi-ate at one season, and disappear at another. Some of the best known e mentioned below. Several investigators have grouped the bacteria und in water into classes according to their biochemical properties. Where groups are subsequently referred to, the classification is that sed by Jordan and followed by many other workers.

B. fluorescens liquefaciens, Group V, together with some closely allied arieteis, is probably more frequently found in water than any other rm, and is easily recognized by the green fluorescence and liquefaction produces in gelatin.

B. fluorescens non-liquefaciens, Group VI, as the name implies does ot liquefy gelatin, but produces characteristic colonies with a fluores-nt shimmer, is often very abundant in river waters, and is representa-ve of a group comprising *B. f. longus*, *B. f. tenuis*. *B. f. aureus*, and . *f. crassus*.

Certain organisms which liquefy gelatin and acidify milk—classed by ordan in his Group VIII—are quite common at certain seasons. me of these are soil organisms and are closely related to the proteus oup; and some of them are *B. liquefaciens*, *B. punctatus*, *B. circulans*.

Chromogenic bacilli and cocci (*Groups XIII, and XIV*) are often esent in water. Of those producing red coloring matter, the well-nown *B. prodigiosus* is the type of the group; others are *B. ruber*, *B.*

indicus, *B. rubescens* and *B. rubefaciens*. Several yellow and orange organisms are commonly found, such as *B. aquatilis*, *B. ochraceus*, *B. aurantiacus*, *B. fulvus*, etc.

At certain times, particulary in river and brook waters, organisms producing violet pigment are quite common. *B. violaceus* or *B. janthinus*, as it is sometimes called, is the prevailing type; others are *B. lividus*, *B. amethystinus*, and *B. coeruleus*.

The chromogenic cocci produce either orange or yellow pigment, and as a rule are not numreous in water. *Sarcina lutea* is the most common species.

Non-chromogenic cocci (Group XV) are more frequent. *M. candicans*, *M. nivalis*, *M. aquatilis*, are non-liquefying forms, and *M. coronatus* is the type of those which liquefy gelatin.

Soil Bacteria from Surface Washings.—During times of flood high water, and after rains, numerous soil organisms are found in natural waters; and occasionally certain species persist for a considerable time. Among the commonest species is *B. mycoides*, with its characteristic rhizoid colony; also *B. subtilis*, *B. mengatherium*, and *B. mesentericus vulgatus*, with its allied varieties; likewise *B. m. fuscus* and *B. m. ruber*— all belonging to Jordan's Group VII, and having many characters in common, such as characteristic colonies, followed by liquefaction when growing in gelatin, production of spores, etc.

Cladothrix dichotoma, one of the thread bacteria, easily recognized on gelatin plates by the brown halo that surrounds the colony, is often found in fresh and stagnant water, and in most soils. It seems to flourish wherever there is much organic matter.

These are the soil organisms most often found when beef peptone gelatin is used for isolating purposes; but if other media are used, a different flora appears, and we find nitrifying organisms, yellow chromogens, etc.

Intestinal Bacteria, Usually of Sewage Origin.—*Proteus Group*.—There are several groups of sewage organisms found in impure water; some of these are very abundant in crude sewage, but are not found in such relatively large numbers in contaminated water. Jordan's Group III contains the organisms belonging to the large proteus group, the principal species being *B. vulgaris*, *B. zenkeri*, *B. mirabilis*, *B. zopfii*, the sewage proteus of Houston, and *B. cloacæ*. All these are frequently found in impure water, and in sewage. In the latter Hou

n has found as many as 100,000 per c.c. All these organisms are mo-
e, liquefy gelatin, and produce gas in dextrose and saccharose broth,
d little or none in lactose; reduce nitrates, curdle milk, produce indol,
d give a fecal, disagreeable odor in broth or other media.

Sewage streptococci.—The streptococci found in sewage are probably
milar to those found elsewhere; but their appearance in contaminated
ter may be regarded as indicative of recent sewage contamination,
cause the bulk of the evidence available seems to show that they are
licate organisms, which rapidly die outside of the body. While it is
sy to ascertain their presence in polluted water, it is almost impossible
enumerate them; and they do not furnish such good evidence of sew-
e pollution as the colon bacillus. They may be said to furnish valu-
le confirmatory evidence of sewage contamination.

B. enteritidis sporogenes.—This resistant, spore-bearing organism is
sually present in the intestinal tract of man; is found in sewage, milk,
d dust; and occurs in foodstuffs, such as wheat, oatmeal, rice, etc.
n account of its ubiquity and the resistance of its spores, it cannot be
nsidered a good indicator of excretal pollution.

B. coli.—The presence of this organism in potable water is gener-
ly accepted as the best bacterial indicator of sewage pollution. It
ust be remembered, however, that there are many varieties of this
rganism, to which certain investigators have given specific names, even
hen the differences from the type organism have been very slight. It
ay be well to mention some of these, to avoid confusion in the mind of
e reader. The true colon bacillus, *B. coli*, or *B. coli communis*, or *B.*
li communis verus, is a short bacillus with rounded ends, motile, forms
o spores and is Gram negative, does not liquefy gelatin, produces
cidity and coagulation in litmus milk, gives rise to acid and gas in
lucose and lactose media, causes canary-yellow fluorescence in neutral
d media, and produces indol when grown in peptone water. The term
Excretal B. coli" has been suggested as a convenient designation of an
rganism which possesses the above characteristics.

A saccharose fermenting variety of *B. coli* has been named *B. com-*
unior; and we have a whole series of organisms which differ more or ess
n various biochemical reactions, or lack some of their positive reactions.
o some of these the name "para-colon" has been given; and the name
para typhoid" has been applied to those which more closely approxi-
ate to the cultural peculiarities of the typhoid bacillus.

For practical purposes in the analysis of water, these distinctions are unnecessary.

Bact. lactis aerogenes, a short, thick, capsulated, non-motile bacterium related to *B. coli*, is also an intestinal organism, and must be regarded as an indicator of sewage pollution.

B. typhosus.—Very few instances are recorded in bacteriological literature of the direct isolation of the typhoid bacillus from infected water. The organism is not long-lived, even in pure water (eight or ten days); and when exposed to the action of sewage bacteria, its longevity is greatly diminished (not more than five to six days). A few resistant specimens may remain alive for longer periods of time.

Although the typhoid bacillus has been found so infrequently in water, it is well understood at the present time that the purification of the water supply of a town or city produces a marked decrease in the number of cases and in the mortality from typhoid fever, as the following table shows: (See also Fig. 110.)

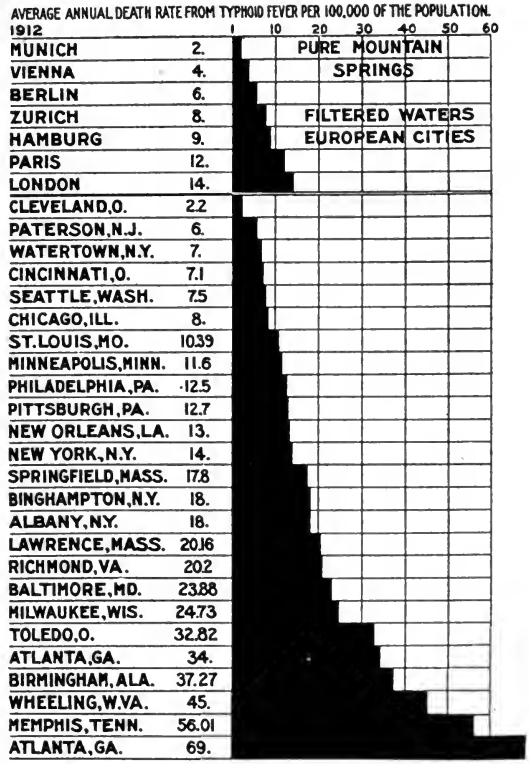
DEATHS FROM TYPHOID FEVER PER 100,000 PER YEAR

Place	Purification by	Date of change	Five years before change	Five years after change	Percentage of reduction
Hamburg.....	Filtration	1892–3	47	7	85
Zürich.....	Filtration	1885	76	10	87
Lawrence, Mass.....	Filtration	1893	121	26	79
Albany, N. Y.....	Filtration	1899	104	28	73

Not only has such a marked improvement followed the purification of public water supplies in the case of typhoid fever, but it has been shown by statistics that "where one death from typhoid fever has been avoided by the use of better water, a certain number of deaths, probably two or three, from other causes have been avoided."

In the routine examination of water, no particular effort is made to isolate this organism, owing to the difficulty of the task. The tests that the present-day investigator has to satisfy are extremely thorough; and unless the suspected organism conforms to the whole of these necessary tests it cannot be accepted as true *B. typhosus*.

Msp. comma.—The spirillum, or vibrio, of Asiatic cholera is an intestinal organism; and the disease it produces is spread largely by water. Epidemics of cholera are more easily traced to the



An instructive contrast between Altona and Hamburg before the latter filtered its water, having learnt its lesson from a sharp outbreak of cholera.

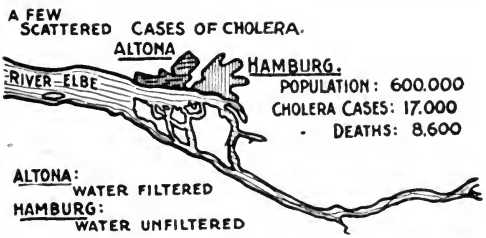


FIG. 110.—(*After G. E. Armstrong.*)

source than those of typhoid fever, owing to the "explosive" character of the disease. At the time of the outbreak of cholera in Hamburg, in 1892, the cholera vibrios were frequently isolated from the water of the river Elbe, which was used to furnish the regular supply of the city. The adjoining city of Altona also obtained its water from the same river, after it had received some of the Hamburg sewage; yet it remained practically free from the scourge, owing to the efficiency of sand filters which were used to purify the water (Fig. 110). In times of epidemic, the organism has been isolated from rivers, wells, and reservoirs in India, a country in which the disease is endemic.

The Number of Bacteria in Rain, Snow, Hail, Etc., and in Water from Wells, Upland Surface Waters, Rivers, and Lakes

Rain.—The number of bacteria found in rain depends upon the month of the year and the dryness of the air. When considerable dust is present in the air, the first rain beats it back to the soil; and at such time rain water contains more organisms than usual. Rain falling in densely inhabited cities always contains more microbes than rain falling on open farm land or upland pastures. A few figures will be sufficient to illustrate.

Number of Bacteria per Liter of Rain Water

Figures for Montsouris Park, Paris, France, and the average for two years

Month	Number of organisms per liter	Month	Number of organisms per liter
January	8,000	July	5,600
February	1,320	August	8,300
March	2,920	September	5,770
April	2,140	October	3,220
May	2,440	November	3,250
June	5,600	December	4,330

Yearly average 5,300 per liter per month.

The average for the interior of Paris corresponds with the larger amount of dust in the air, and reaches a total of 19,000 organisms per L. With a yearly rainfall of 609.6 mm. (24 inches), the rain washes down during the year some 5,000,000 organisms to the square yard.

Snow.—The results obtained from snow are similar to those obtained from rain; but as a rule the numbers are larger, a result doubtless due to the larger particles of the snow flakes. One investigator has found from 334 to 463 bacteria per c.c. of snow water. On the summit of high mountains snow is practically sterile, Binot not finding a single organism in 8 c.c. of water from mountain-top snow.

Water issuing from glaciers is of remarkable purity, containing only from three to eight organisms per c.c.; but the numbers are larger as the distance from the glacier increases.

Hail.—Hail stones usually contain large numbers of bacteria, varying from 628 to 21,000 per c.c. of water obtained from the melting hail. Fluorescing bacteria have been found in some samples; and the presence of these microörganisms suggests that surface water is sometimes carried up by storms and congealed. The presence of many molds in hail is due to contamination from the air.

Deep Wells.—Deep well water and spring water contain as a rule but few organisms, usually less than 50 per c.c. on gelatin at 20°, and less than 5 per c.c. on agar plates at blood heat. In a series of tests of water taken direct from forty-three artesian wells, 152.4 M. (500 feet) deep or more, the writer has found an average of 27 per c.c. for the gelatin and 1.5 per c.c. for the agar counts. These tests have extended over a period of several years; and water from deep springs has given similar results.

Shallow Wells.—The bacterial content of shallow wells depends greatly on their location and construction. Even in those well located and constructed, the number varies with the amount of rainfall, and is often large. In polluted wells, very high numbers of organisms are found.

Sedgwick and Prescott found from 190 to 8,640 bacteria per c.c. in unpolluted wells.

In the same class of wells, Savage found from 10 to 100 per c.c. by the blood-heat count, and 100 to 20,000 or more by the gelatin count.

Sixty polluted wells examined by the writer gave an average gelatin count of 740 bacteria per c.c.; and thirty-eight wells which were free of contamination gave an average count of 400 per c.c.

Polluted wells often give counts approximating the higher numbers mentioned above; but, of course, the character of the bacterial flora is quite different.

Upland Surface Waters.—There are few bacteria in upland surface waters draining barren uplands. Cultivation, grazing of animals, and human habitation produce other conditions. In pure waters, 50 to 300 per c.c. by the gelatin and 1 to 10 by the agar count are found.

Rivers.—The greatest variation in the number of bacteria exists in river waters. Many factors, such as sewage contamination, temperature, rain fall, vegetable débris, etc., influence the microbial population. A few figures may be given for illustration.

Bacteriological Examination of Rivers at and below Large Sources of Pollution (Boyce and Co-workers)

Distance	Direction	Munich. River Isar	Cologne. River Rhine
	Above	305	4,786
About 0.6 mile	Below	9,387	
About 2.7 miles	Below	13,503	
About 6.0 miles	Below	8,764	30,432
About 12.0 miles	Below	4,796	12,460
About 15.0 miles	Below	3,602	9,595
About 26.0 miles	Below	.....	7,869

In the Chicago drainage canal, Jordan found 1,245,000 bacteria per c.c. at Bridgeport; 650,000 at Lockport, twenty-nine miles below; and 3,660 at Averyville, 159 miles below. Below where the sewage of Peoria enters, the number rises to 758,000 at Wesley City, and decrease to 4,800 at Kampsville, 123 miles from Peoria.

The River Rhône contains an average of 75 bacteria per c.c. above Lyons and 800 below. The Dee, 88 above Braemar and 2,829 per c.c. below. Many more similar results are found in the literature.

Lakes.—The water of lakes is generally much purer than river water. Near the shore, the bacterial content is higher than farther out, showing the contaminating influence of habitation. Thus Lake Geneva contains as many as 150,000 bacteria per c.c. near the shore and further out only 38 per c.c. Other figures are as follows: Loch Katrine, 74 per c.c., Lake Lucerne, 8 to 51 per c.c., Lake Champlain 82 per c.c.

Sea Water.—There are few bacteria in sea water remote from the coast; but near the shore and in the neighborhood of seaports there may be large numbers.

Examples: 350 M. from Naples, sea water contained 26,000 bacteria per c.c. At a distance of 3 KM., only 10. Samples taken from depths of 75 to 800 M. at distances from 4 to 15 KM. from shore were found to contain from 6 to 78 bacteria per c.c. in surface water, and from 3 to 260 at various depths below.

Causes Affecting the Increase and Decrease of the Number of Bacteria in Water

There is a number of causes which influence the multiplication or diminution of microörganisms in natural waters; and while it is necessary to discuss each of these causes in detail, it must be remembered that a number of them may be simultaneously influencing the increase or decrease.

Temperature.—In natural waters, a low temperature probably acts injuriously on parasitic bacteria, reducing their numbers; but the bacterial content of water during the hot summer months is generally not so large as during the cooler seasons. Water collected for examination should be analyzed at once; otherwise, contradictory results as to numbers will be found. Usually, in most waters, there is a reduction in numbers for a few hours, followed by a large increase. Very much polluted waters, however, show a marked decrease of intestinal organisms, if the samples are kept cool.

Light.—Although the germicidal effect of sunlight is well known, yet it has not such powerful effects on the bacteria in water. Much depends, no doubt, on the turbidity and speed of the current, the maxmium killing effect being produced in shallow, clear and slow-moving water. It has been found by experiment that the germ-killing power of light extends to a depth of 3 M (about 9.84 feet). As a means of purifying water, direct light produces very little effect.

Food Supply.—The amount of organic matter in water directly influences the growth of bacteria. Where a large amount of this is present, the number of microörganisms is also large. Rivers containing considerable organic matter derived from vegetable débris, etc., contain, as a rule, more organisms than rivers in which there is but little of such material. Thus the Ottawa River, which drains a large area of forest lands and is characterized as an upland peaty water carrying a rather high percentage of organic and volatile matter, contains through-

out the year a larger number of organisms to the cubic centimeter than the water of the river St. Lawrence, which is much clearer and contains much less organic matter. Sewage water is rich in organic matter, and proportionately rich in bacterial life; and bacterial purification is synchronous with a diminution of organic matter.

Jordan remarks in this connection that "in the causes connected with the insufficiency or unsuitability of the food supply is to be found the main reason for the bacterial self-purification of streams."

Oxidation.—On the surface of waters, in rapids, falls, and tidal rivers, much oxygen is absorbed, and much impure matter is oxidized. Such oxidation is one of the minor agencies in the purification of water.

Vegetation and Protozoa.—Low forms of plant and animal life, like certain species of algæ, river plants, and the numerous protozoan forms, bring about a reduction of organic matter in water, and thus reduce the amount of food available for bacteria. There is also the antagonism between these forms and bacteria. The chemical products of the higher forms are considered by some authorities to be injurious to bacterial life; and many bacteria are ingested by predatory protozoa.

Dilution.—Sewage flowing into a river or lake is at once diluted with quantities of pure water, and the amount of available food material is thus diminished; the space occupied by a definite number of bacteria is increased; and it is easy to see that the greater the dilution, the fewer sewage bacteria will be found. An example will suffice to illustrate. The sewage of the city of Ottawa amounts to about 454 L. (100 gallons) per second; and the gelatin count from it gives an average in round numbers of 3,000,000 bacteria per c.c. The yearly mean discharge of the river is about 1,364,511 L. (300,000 gallons) a second; and thus the sewage becomes diluted 3,000 times.

Sedimentation.—Impurities, suspended matter, and bacteria having weight, naturally gravitate to the bottom; and the subsidence of these matters is spoken of as sedimentation.

Lake water being still, sedimentation in it is more marked than in moving water; and such water contains but few bacteria. In slow-moving rivers the influence of this factor is also quite pronounced; and, according to Jordan, "The influences summed up by the term *sedimentation* are sufficiently powerful to obviate the necessity for summoning another cause to explain the diminution in numbers of bacteria" in sewage polluted rivers. The example already given

the self-purification of the Chicago drainage canal illustrates Jordan's ntention.

Other Causes.—There is a number of other causes, not well nown nor of sufficient practical importance for more detailed com- ent, which may increase or decrease the number of bacteria in water, ch as the inhibiting action of microörganisms and their products one another, the effects of pressure, etc.

A peculiar fact, which has never been satisfactorily explained, is the ick death (in three to five hours) of the cholera vibrio in the waters the Ganges and Jumna. When one remembers that these rivers e grossly contaminated by sewage, by numerous corpses of natives ften dead of cholera), and by the bathing of thousands of natives, seems remarkable that the belief of the Hindoos, the water of these vers is pure and cannot be defiled, and they can safely drink it and lthe in it, should be confirmed by means of modern bacteriological search. It is also a curious fact that the bactericidal power of mna water is lost when it is boiled; and that the cholera vibrio opagates at once, if placed in water taken from wells in the vicinity the rivers.

Interpretation of the Bacteriological Analysis of Water

In making any analysis of water, all data, such as the kind of ter and the particulars regarding collection, transmission, sampling, infall, etc., should be given, as these are a great help in interpreting e results. One analysis is rarely sufficient; examinations should regularly and systematically made.

Quantitative Standards.—No absolute guide can be given to termine the potable quality of water from the number of micro- ganisms in it. It may, however, be safely assumed that high bacte- l counts indicate a large amount of organic matter. The number of ganisms growing in beef peptone gelatin at 20° to 22°, and termed e "gelatin count," should be given. For deep wells and springs, is should not exceed 50 per c.c.; and for shallow wells and rivers, t over 500 per c.c. After rains or floods, these figures might be ceeded, and would not necessarily indicate dangerous pollution.

The number of organisms which develop on beef peptone agar ubated at blood heat, commonly termed the "agar" or "blood-

heat" count, is perhaps more important than the gelatin count, as many water bacteria do not grow at blood heat, whereas sewage and soil organisms grow readily at this temperature. The agar count eliminates the water *flora*, but obscures the sanitary results by reason of the presence of soil bacteria. For deep waters, the agar count should generally not exceed 10 per c.c.; and for surface waters, not over 100 per c.c.

Qualitative Standards.—The isolation and identification of specific disease organisms, such as typhoid and cholera microbes from water, is sufficient to condemn such a sample as unfit for use, but, on account of many technical difficulties, it is practically impossible to make such an examination. Apart from a few special cases, where it may be necessary to attempt the isolation of these pathogenic bacteria, the presence of the colon bacillus (*B. coli*) in small amounts of water, is generally looked upon as significant and indicative of sewage pollution. The technical methods used in this isolation and enumeration are many, and may be found in the works cited; but there is considerable difference of opinion as to the *number* of *B. coli* which should condemn a sample of water. Prescott and Winslow state that if the colon bacillus is in "such abundance as to be isolated in a large proportion of cases from 1 c.c. of water, it is reasonable proof of the presence of serious pollution." Savage suggests that *B. coli* should be absent from 100 c.c. in the case of water from deep wells and springs, and should be absent from 10 c.c. in surface waters, such as rivers used for drinking purposes, shallow wells, and upland surface waters.

The streptococcus examination is next in importance as an indicator of sewage. Streptococci should be absent from the amount of water mentioned above for *B. coli;* and *B. enteritidis sporogenes* should not be present in 1,000 c.c. of water from deep wells, nor in 100 c.c. from surface waters.

Sedimentation, Filtration, and Purification of Water

As areas become more and more thickly settled and towns and cities increase in population, the problem of obtaining sanitary control over the water supply increases in importance. Very few towns and cities are fortunate to obtain their water supply from an unpo

hed area. Consequently expensive installation must be made, in order tpurify a suspiciously contaminated water by freeing it from organisas injurious to health. There are several methods of accomplishin such purification; and these will be briefly mentioned.

Sedimentation and Filtration.—This method of purifying water hs been used for nearly a hundred years; but the great impetus given to ts hygienic measure was due to Koch, who showed in 1893 that the

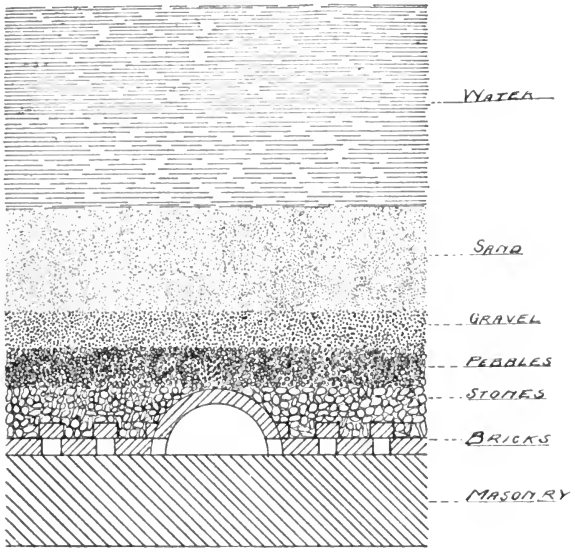


Fig. 111.—Section of a sand filter.

pper filtration of Elbe water saved the town of Altona from an epidmic of cholera which devastated Hamburg as a result of drinking unfiered water. In this system of purification, the water is first stored in lge reservoirs, where the effect of sedimentation and storage reduces csiderably the number of bacteria. From the reservoir, the water is fiered through sand, gravel, and pebbles, etc., arranged as shown iFig. 111. This filtration removes from 97 to 99.5 per cent of the rcroörganisms.

The action of the fiter bed is due to the mechanical obstruction of ipurities, to oxidation of the organic matter, and to nitrification due

MEAN OF MONTHLY EXAMINATIONS FOR THE YEAR

	Microörganisms per c.c.		
	At source	After storage	After filtration
London, Lambeth Works.....	16,138	7,820	75
London, Chelsea Works.....	16,138	1,067	34
Berlin, Lake Müggel.....	1,400	.....	60
Paris, Marne.....	79,000	.....	630
Paris, Seine.....	186,986	.....	400

to the living bacteria in the scum which forms on the top of the layer sand. Of these, the last is the most important; for until this gelatino layer forms, the filter does not act properly—in fact, it has little filte ing action, as the following figures show:

BACTERIAL CONTENT OF WATER BEFORE AND AFTER CLEANING THE SAND FILT

Before cleaning, *i.e.*, before removing the scum layer...	42 per c.c.
One day after cleaning.....	1880
Two days after cleaning.....	752
Three days after cleaning.....	208
Four days after cleaning.....	156
Five days after cleaning.....	102
Six days after cleaning.....	84

Thus provision must be made to permit the scum or film to form b fore the filtered water is used for domestic purposes.

The rate of filtration must be regulated; for if the water is allowed exceed a certain rate (101.6 mm. or 4 inches per hour), inefficien follows.

COAGULATING BASINS AND FILTRATION.—This method of purific tion consists in adding a coagulant, such as basic sulphate of aluminu by means of a mechanical device which regulates the quantity, as t water is pumped into the coagulating basins or reservoirs, where it r mains for six to twenty-four hours. The aluminum sulphate is deco posed by the lime in the water and forms insoluble aluminum hydrat and the sulphuric acid combines with the lime. The hydrate of alum num is precipitated in large flocculent masses, entangling all particl of soil or organic matter; and these, being deposited on the surface of t

sad, form the filtering layer. Such filters are very efficient; they remove from 97 to 99.8 per cent of the bacteria from the water.

Porous Filters.—(Fig. 112.) These filters are either made from unglazed porcelain or baked diatomaceous earth; the former are known as Chamberland, and the latter as Berkefeld filters. These filters are usually candle-shaped, require considerable pressure to force water through them, and can be used only when a small supply of water is needed. Water which is forced through these filters is at first sterile; but with repeated use they allow bacteria to pass through the pores and

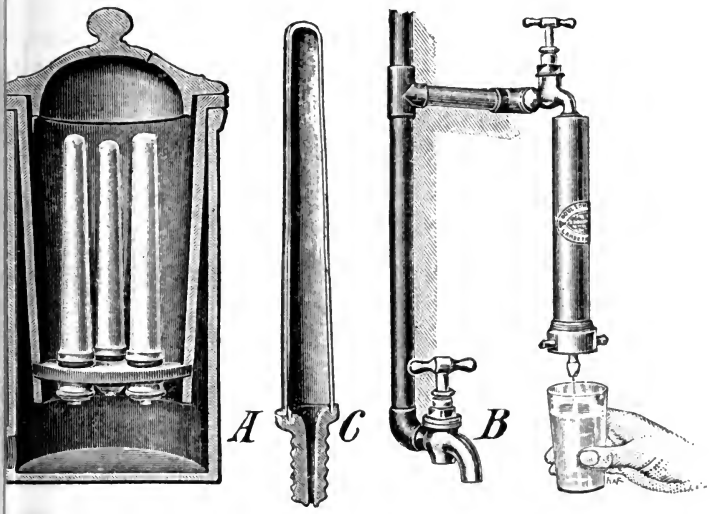


Fig. 112.—Unglazed porcelain filters. Chamberland system; *A*, without pressure; *B*, fitted to main water supply; *C*, section of a porous porcelain filter.

thus the filtering efficiency is impaired and will remain so, until the filters are cleaned and baked to red heat in a muffle-furnace. Unless this is done regularly, no dependence should be placed on these filters, as they only put those who use them off their guard against the danger to which they are exposed.

Purification by Ozone.—The antiseptic properties of ozone are well known. It is used in the purification of the water supply of some towns—Nice, Chartres, etc. Ozone used for this purpose is usually obtained by means of the electric current; and a flowing film of water is

brought into contact with an upward current of air charged with ozone which current makes the water almost completely sterile. This metho of purification is efficient, but rather expensive.

Purification by Heat.—By bringing water to the boiling point, a harmful bacteria are destroyed; a few spores may resist this treatmen but they are harmless. Boiled water is of a flat, insipid taste, due to th driving out of the contained gases. The taste may be improved b cooling and shaking. The boiling of water is often resorted to as a hy gienic measure in times of epidemic, and for the supply of armies in th field.

Purification by Chemicals.—The addition of a small amount c calcium hypochlorite, or potassium iodide, etc., purifies water; but thes methods are seldom used, except for the use of soldiers on campaig Hypochlorite, however, is now used more commonly in municipal wate supplies where they can not be otherwise controlled.

Location and Construction of Wells

Farms in many sections of this country are practically all supplie with surface water collected in shallow wells. Hence farmers shoul understand the principles involved in the location and constructio of wells.

Many farm wells are badly located—too near such sources of co tamination as outhouses, cesspools, stables, or barnyards; and tho who locate them give too little attention to the slope of the ground, ar the nature and slope of the subsoil. There should be at least 22 30 M. (75 to 100 feet) between the well and all probable sources contamination; and this distance is too small, if the soil is very porou or if the surface and subsoil drainage is toward the well, or if the w is sunk in fissured rock—as it is obvious that there are serious chanc of contamination in each of the above circumstances.

In all cases, the surface drainage should be away from the well; an as far as possible, the subsoil drainage also should be *from* the well.

Sketches 113, 114, and 115 illustrate these points, the upper part each drawing showing the plan and the lower portion a section throug the dotted line marked on the plan. Fig. 113, shows that the surfa drainage is from the house, privy, stables, and barnyard toward the we The section through the line "A" shows the relation of the impervio

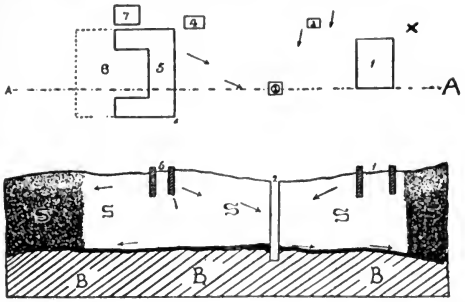


FIG. 113.

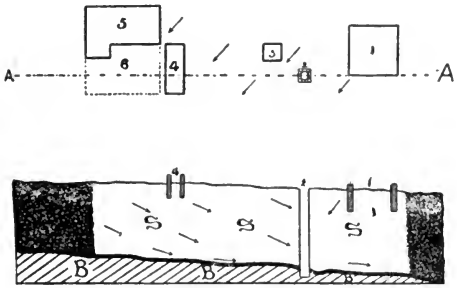


FIG. 114.

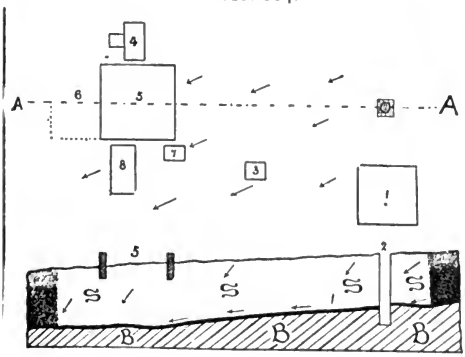


FIG. 115.

FIGS. 113, 114, and 115.—In each figure—plan above—section through A B below. S = Soil; B = Impervious subsoil or strata. 1, House; 2, well; 3, outhouse; 4, piggery; 5, stables; 6, stable yard; 7, hen house; 8, sheep stable. Arrow heads indicate direction of water flow. (Original.)

subsoil "B" to the drainage. Water falling on the surface of the ground would penetrate through the soil to the upper portion of the subsoil, and then move along it in the direction of the greatest slope. In this sketch, the subsoil drainage is away from the well; and in this respect the well is located properly; but, in respect to the surface drainage, improperly located. A better place for the well would be at the letter "X".

In Fig. 114 the surface drainage—including that from the adjacent outhouse at 3, which is too close to the well—is toward the barn and away from the well; but the subsoil drainage from all the buildings

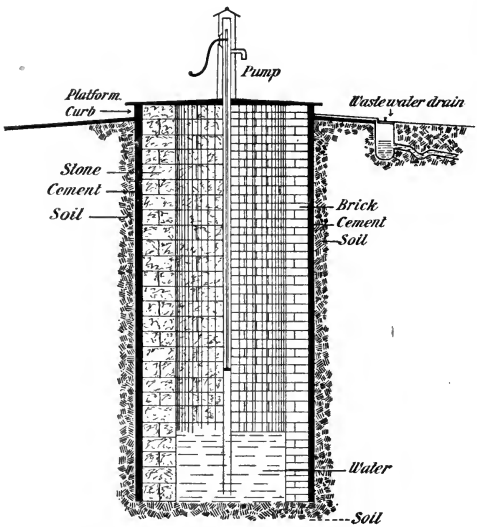


FIG. 116.—Construction of a model well. On the right is brick construction, o the left stone construction, as illustrated. (Original.)

except the house, is in the direction of the well; and thus contaminatio of the water supply is liable to occur.

Fig. 114 shows a well properly located as regards both surface an subsoil drainage. Such a well will supply pure water, if it is properl constructed.

Fig. 115 shows the proper construction of a well with brick or stone Large vitrified drain pipes with cemented joints will answer equally wel when there is an abundant supply of water; but in case the supply o

wter is limited, a large area is needed, and a stone or brick well is nessary.

Reference to the illustrations will show that every endeavor is made torevent surface water from entering directly into the well. The walls ar impervious; and the earth or clay is well rammed against the outer sie of the wall. The curb is carried well above the surface of the grund. The waste water is conducted by means of a sloping platform, trp, and drain, away from the well; and the well opening is properly cdered. All water entering such a well must percolate through a consierable depth of soil, and undergo purification by means of the aggregions of living bacteria in the soil spaces. Thus the soil around a well fuils the same function in purifying the surface water as the scum laer that forms on the surface of gravel filters.

CHAPTER II*

MICROBIOLOGY OF SEWAGE

The Bacterial Flora of Sewage

Complexity of Flora.—Sewage is made up of the miscellaneo and varied wastes of human life and activity, and the bacteria which a found therein are the result of a haphazard and chance admixture substances of diverse origin and character. The resulting flora is n only of great diversity and variability, but it is with few exceptions no characteristic. In brief, the medium with which we have to deal h had an origin too indefinite and a history too short to have permitte the establishment of anything approaching a constant or characteris bacterial flora.

Typical Forms.—Our interest in this sewage flora is a very practic one, being confined to those organisms which carry on the work of bi logical purification and to certain pathogens which for obvious reaso require special treatment. We are interested chiefly in what these ba teria do rather than in what they are, and our classification is influenc accordingly. It is based, not upon the species or the genus nor eve upon the group or type, that proves so convenient in general bacteri classification, but upon a sort of physiological or functional type, havi to do solely with the activities of the organisms in sewage and in its pu fication. Bacteria performing a common function or producing a co mon result are members of one type. Individuals may belong to sever of our types and there are doubtless a great many that belong to no These latter simply have no place assigned them as yet in the rôle sewage purification, because they possess none of the recognized typic functions.

Apparent exception may be taken to these general principles the case of such organisms as the *B. coli*, sewage streptococci a *B. enteritidis*. These are, to a certain extent, characteristic sewa bacteria. But interest in them as individuals is confined to wat

*Prepared by Earle B. Phelps.

bacteriology. If they have any functions in the bacterial changes of sewage, they receive attention as members of a corresponding type, not as individuals. A study of these sewage types, therefore, is a study of the chemical changes induced in the medium by the activities of one or the other group of bacteria.

Types of Sewage Bacteria

According to the general character of the changes which they bring about, sewage bacteria are divided into two large groups, the anaerobic or putrefactive bacteria, and the oxidizing bacteria. In regard to the former, no attention is paid to the fine distinctions that have been made in recent years in connection with the definition of putrefaction. In sewage chemistry putrefaction is that change which takes place naturally in sewage after anaerobic conditions have become established. It involves the reduction of urea, the hydrolysis of protein and of cellulose, the emulsification of fats, the reduction of nitrates and sulphates and possibly of phosphates, and those other changes which are characterized by the withdrawal of oxygen and the hydrolysis of complex molecules. These changes are always noted in sewage under anaerobic conditions and the terms putrefactive and anaerobic change are for the present purposes practically synonymous.

The oxidizing reactions on the other hand might be classed under the general heading of aerobic reactions, except that they constitute only a small portion of the group of reactions which take place normally under aerobic conditions. They are distinguished by the fact that oxygen is added to the molecule, the product always containing more oxygen than the initial substance. Carbon dioxide, water and nitrates are produced, in distinction from methane, hydrogen and ammonia, which characterize the anaerobic reactions. A third type, possessing objective rather than subjective functions, in sewage, is made up of pathogenic and other harmful bacteria. These play no part in our theories of purification and the proof of their presence is generally lacking. For the protection of the public health, it is assumed that they are always present in sewage, and our procedure in sewage disposal is modified throughout in accordance with this assumption.

With these definitions in mind we may proceed to a more detailed study of the bacterial types themselves.

Putrefactive and Anaerobic Bacteria.—Putrefaction or anaerobic fermentation involves the withdrawal of oxygen from one molecule or part of a molecule and the subsequent oxidation of another molecule or part of the same molecule. The energy released in this process is utilized in the vital functions of the organism. This action is neither oxidation nor reduction, or more strictly, they are both taking place simultaneously.

A good example of such a process is the fermentation of urea. The reaction takes place as follows:

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3.$$

Carbon is oxidized at the expense of hydrogen, a process which, by itself, is endothermic, that is, requires heat or energy for its maintenance. But the heat of formation of the final product is greater than that of the initial substances and the energy thus liberated becomes available for use by the bacteria. It is in this way that hydrolytic changes of this character play the same rôle in anaerobic reactions that is played by direct oxidation under aerobic conditions.

The Liquefaction of Protein.—One of the most clearly defined and useful types of bacterial activity to be seen in the various sewage disposal processes is that which we term liquefaction. This term is used to denote broadly all those changes by which solid and insoluble organic matter is converted into a soluble condition. The particular process known as protein liquefaction is in the main analogous to gastric digestion. Its one characteristic is the increased solubility of the product. The practical importance of protein liquefaction in sewage disposal is very great and the value of the liquefying bacteria correspondingly high. Nevertheless, aside from our knowledge of analogous processes in digestion and in bacterial putrefaction of albuminous substances, we know almost nothing of the chemistry or the bacteriology of this process. An enormous variety of bacteria are included in this group. The whole process is doubtless the result of a very complicated symbiosis in which various sub-groups of bacteria carry out the initial reaction, from which point other groups carry out the initial reaction. from which point other groups carry it through successive stages. Absence of one or another of these groups or of some important species of any group doubtless accounts for the diverse results that are recorded. It is well known that the activities within a septic tank, for example,

are seldom twice the same. Gross differences readily apparent to the senses of one versed in such matters certainly exist, and in actual results it is rare to find two tanks doing exactly the same kind of work. Much depends of course upon the chemical character of the sewage itself, but much, that is still unexplained, must eventually be traced to the great diversity of the sewage flora and the complex symbiosis as well as bacterial antagonisms that are involved in the reactions with which we are dealing.

During these reactions proteins and albumins are hydrolyzed by seccessive stages to albumoses, peptones, amino-acids, amines, and finally to ammonia, carbon dioxide, methane, hydrogen, etc. Simultaneously ammonia, amines, and carbon dioxide are eliminated at each stage as products. The tendency then is toward simple, soluble and gaseous side products, and hence of value in the preliminary resolution of the sewage.

The Fermentation of Cellulose.—The fermentation of cellulose is, next to protein hydrolysis, the most important work of the anaerobic bacteria in sewage treatment. So far as is definitely known this action is usually confined to anaerobic conditions. The fact that fence posts decay first at the surface of the ground, or that wood in general decays more rapidly when it is exposed to only a slight degree of moisture, than when it is immersed in water is only an apparent contradiction. The conditions are aerobic in both cases and aerobic bacteria would not be favored by total immersion but the effect in both instances seems to be due to fungus growths which are more active in the moist wood.

The anaerobic fermentation of cellulose is that which is found typically in marshes and of which the chief products are carbon dioxide and methane or "marsh gas." Nitrogenous food material is also requisite, which accounts for the preserving property of reasonably pure water upon wood.

In the septic tank the solution of cellulose is extremely rapid, and large pieces of cotton cloth or rolls of paper are completely dissolved within a few months. Wood itself is more resistant and withstands the action of the tank for years. This is largely due to the fact that the wood molecule is much more complicated than a simple cellulose molecule, and, among the conifers at least, to the further fact that antiseptic intercellular substances are present.

Chemically considered the action is hydrolytic and can be imitated

by prolonged boiling in dilute acids. Pectin substances, starches and finally sugars are produced while butyric and other organic acids, carbon dioxide and methane appear as by-products. Bacteriologically, although it has variously been ascribed to one or another organism, it is probably the result of the activities of many and is possibly not the principal activity of any one of these. In other words, cellulose fermentation is probably a series of side reactions produced during the fermentation of the nitrogenous material rather than a definite reaction upon which the metabolism of any single species depends. This view is strengthened by the general observations that this fermentation is in most cases due directly to enzymes. Viewed in this light it is easy to understand the difficulty that has surrounded the isolation of definite cellulose fermenting organisms. Many have been described, chief of which are *B. butyricus* or *B. amylobacter*, *B. omelianski*, *Sp. rugula.*

The Saponification of Fats.—A third great group of type reactions occurring under anaerobic conditions is the saponification or splitting of fat. Our knowledge of this process is even less definite than of the cellulose fermentations. It is a fact that there does take place in sewage a gradual saponification and emulsification by which the fat loses its identity and mingles with the liquid. This effect is most noticeable in the case of long sewers in which considerable velocities are maintained. In quiescent tanks there is a tendency for the fats to rise to the surface and thus become removed from the influence of this action. Thus in small installations enormously heavy scums form upon the tanks and analysis shows a considerable percentage of fat in this material. In larger systems on the other hand there is less and less evidence of fatty material as such. It is true that there is a deposit upon the walls and tops of such sewers and that small floating objects, like matches, rolling along such a wall will accumulate layers of grease and become eventually the familiar "grease-balls" found in the discharge, but in the main the fatty material has become well disintegrated before the outlet is reached.

In this case also as in that previously discussed it is not believed that the action is a direct result of the activity of any particular organism. The proteolytic changes are accompanied by the freeing of alkaline products, ammonia and amines, which leads to some saponification, and which, in turn, leads to a further emulsification. It has also been demonstrated that bacterial activity is commonly associated with fat

saponification and decomposition. Whether specific enzymes are present which assist in this final process or not has never been determined. It is significant to note, however, that where sewages are slightly acid, unaltered fats are much more abundant, even though the acidity is insufficient to prevent vigorous putrefactive changes in the sewage itself.

The Fermentation of Urea.—The fermentation of urea has already been referred to as a typical and simple case of anaerobic decomposition. This reaction has great significance in sewage chemistry since a considerable proportion of the nitrogen of sewage is present initially as urea. Owing to the ease and rapidity with which the reaction takes place, however, no special effort is necessary to bring it about in sewage treatment and it therefore receives brief attention in discussions of the chemistry of sewage. The change to ammonia takes place in the small sewers of the system and it is difficult and generally impossible to detect the presence of urea in sewage. It has even been suggested that certain enzymes present in fecal matter are instrumental in bringing about this change and that the bacteria are only indirectly concerned. It is known, however, that a large number of bacteria of general occurrence have the power to produce this fermentation. Of these the *Bact. ureæ* (Miquel) may be cited as an example.

The Reduction of Sulphates and Nitrates.—The production of sulphuretted hydrogen during the anaerobic decomposition of sewage is commonly noted. This substance may arise in at least two ways. Sulphur, being a constituent of most protein substances, is split off from the molecule in this form during certain types of fermentation. This formation in these cases is analogous to that of ammonia from protein. The amount so produced is small and is usually neutralized and precipitated by the small amounts of iron and other metals always present in sewage. There is therefore no liberation of the gas itself and it is often said that sulphuretted hydrogen is not formed normally in a septic tank. This conclusion is readily disproved by a simple test of the black residue found at the bottom of such tanks.

A second and more important source of this substance is the sulphate normally present in many sewages. Throughout many parts of the country the water supply contains material quantities of magnesium or calcium sulphate, and upon the sea coast the sewage generally receives more or less salt water.

In these cases the reduction of sulphates to sulphuretted hydr gen is not only of interest bacteriologically but probably exerts a influence upon all the reactions that are going on simultaneousl In fact this example serves excellently to illustrate the great comple ity of these anaerobic reactions and the mutual interdependence each upon all the others. Sulphates, under anaerobic condition are a source of oxygen and it is upon oxygen that the course of all the reactions depends. Therefore the presence of sulphates and t possibility of their yielding oxygen may alter the course of the oth reactions involved. The products of the protein hydrolysis for e ample may be profoundly modified by the presence of this addition source of oxygen.

The effect upon the bacteria themselves is also to be consider as a factor quite distinct from the purely chemical effect just d scribed. It has frequently been observed, and in fact would be e pected, that the products of anaerobic putrefaction are themselv detrimental to the activity of the organism producing the chang in question. The nature of sulphuretted hydrogen makes it appe quite probable that we are dealing here with a toxic substance th would at least inhibit the activities of certain bacteria and in this w further modify the final result.

The same might be said of almost all the reactions with which have to deal but this example is cited as a typical one.

It is known in practice that the presence of sulphates in a sewa does lead to a distinct type of anaerobic change which is characteriz by the marked blackening of the sewage, the formation of seconda reaction products which precipitate after the removal of the suspend matter of the sewage, the evolution of hydrogen sulphide, an excessi amount of mineral or non-volatile residue in the sludge and the form tion of free sulphur upon subsequent aeration of the sewage.

Here again, as in the other types of reaction, it is useless for the pr ent to attempt to ascribe this reaction to any particular species. *S desulphuricans* and *B. sulphureus* have been isolated. A non-liquef ing anaerobic bacillus, which reduced sulphates strongly, was isolat from Boston sewage in the writer's laboratory by G. R. Spauldir Others have been described and there is undoubtedly a large group organisms capable of bringing about the reaction.

Just as the reduction of nitrates is a function performed by man

perhaps most, anaerobes, so the reduction of sulphates, although a less common function, is still common to many forms. In fact nitrates, sulphates, and phosphates form a series in regard to their reducibility and the effect of their presence upon the reaction as a whole. The phosphates so far as has been recorded are not ordinarily reduced.

Oxidizing Bacteria. *The Production of Nitrate and Nitrite.*—A long series of investigations upon the organisms which oxidize nitrogen began with the Franklands and Winogradski, and has continued to the present day. These have given us much information concerning the habits and functions of the nitrifying organisms. Winogradski's original types were *Nitrosomonas* and *Nitrobacter*, the former oxidizing ammonia to nitrite, the latter completing the oxidation to nitrate. Work upon these organisms constitutes such an important factor in soil bacteriology to-day that more detailed discussion of this nitrifying function is left for another place.

In the earlier days of sewage purification great stress was laid upon the work of these organisms, which was believed to be fundamental. The degree of nitrification was accepted as a measure of the work of the filters and little thought was given to the possibility of oxidizing reactions by other forms. With the development of modern sewage disposal methods, the work of this latter type of bacteria has assumed a more important rôle and the actual work of the nitrifying organism has been found to be of only minor and incidental importance.

Other Oxidizing Reactions.—The great groups of aerobic and facultative bacteria are in general concerned in the oxidation of organic matter. There is nothing specific in this reaction and very little that is characteristic of any special or smaller groups. Under certain special and restricted conditions, typical products are formed by particular species, as in the manufacture of vinegar, and it is possible that a careful study of the complex reactions involved in the oxidation of sewage would show a certain sequence in the order of events and certain definite work being accomplished by definite groups. In other words, symbiosis and specialization doubtless take place to a limited extent. But the fundamental fact remains that the metabolism of the organism demands that organic matter be oxidized for the production of energy. Even though certain food substances may be preferred and certain

decompositions be normally produced there is necessarily a great latitude and great adaptability.

For this very reason a study of the individual organism and its action upon specific materials throws no light upon the major problem, which is, given fifty different types of organisms and fifty different fermentable substances, in a mixture, what will be the course of the reaction? Here the preferences, the adaptability and the antagonisms all come into play and while it is impossible to say what has happened or how, it is readily conceived and, in fact, almost apparent, that out of this heterogeneous mixture there will come a homogeneous symbiotic family and an orderly sequence of chemical events, in which metabolic needs and food supply are all delicately adjusted.

Pathogenic Bacteria. *Prevalence and Longevity.*—Owing to its origin and nature, sewage may at any time contain infectious material and for the purposes of the sanitarian it is assumed that at all times the germs of disease are present. Such an assumption is possibly in excess of the actual facts and is only justified because it supplies the only possible hypothesis having an adequate margin of safety. The actual prevalence of pathogenic bacteria obviously depends in the first instance upon the amount of sickness in the contributing community. Furthermore, if, as we are coming to believe, a definite proportion of the population are perpetual carriers of typhoid infection then to just as definite an extent is the bacterial population of the sewage made up of typhoid bacteria from apparently well persons. In addition to these, about five one-hundredths of 1 per cent of the population of American cities are suffering from the disease in acute form. Making due allowance for the extra precautions that are, or should be taken in the care of the dejecta, these persons constitute a definite and fairly constant source of infection.

In the case of the other infectious diseases of the alimentary tract, and, possibly to a less extent in the case of tuberculosis, diphtheria, and many others, these general statements are equally applicable, so that the possibility of the occurrence of infectious material in sewage is not a remote one, but definite and almost quantitatively determinable.

As to the persistence of active pathogenic bacteria in the sewage for any length of time the data are less exact. In the case of typhoid fever, which has been more carefully studied than any other disease, the germs are more persistent in pure water than in impure, but whether this

rality can be extended to sewage is debatable. Our best informa-
leads to the belief that any reduction in numbers of typhoid
eria which may take place within the sewer before discharge is of
r importance and of slight sanitary significance.

Discussion of other pathogens must be in even more general terms.
rmation is almost wholly lacking and it can only be assumed for
oses of safety that, in so far as organisms of these various types are
harged into the sewer, they will persist to a certain extent in the
ge until it is finally disposed of. If such disposal be by discharge
a stream without purification; then the waters of that stream
me polluted with infectious material. Studies recently made by
wick and McNutt have indicated the possibility that many dis-
s, other than the oft-quoted typhoid fever, may be transmitted
is way.

ife in Septic Tanks and Filters.—With the introduction of the
c tank at Exeter, England, in 1893, the question of the fate of
ogenic bacteria in such a tank was raised. It was even suggested
bacteria, such as the typhoid organism, might multiply in the
. The question was investigated by Professor Sims Woodhead,
concluded that no organisms capable of setting up morbid changes
imals were discharged from the tank. This negative evidence,
ever, has little weight in the light of more recent experiments.
ard introduced an emulsion of typhoid bacteria into this same tank
noted only a gradual decrease. After fourteen days he was able
tect 1 per cent of the initial number. He also reported a removal
o per cent of the typhoid organisms introduced into a contact
t. These data must be interpreted in the light of two established
. The typhoid organism tends to die at a rapid but diminishing
under any but the most favorable conditions. This results in a
decrease at first, with a prolonged survival of a few individuals.
process takes place in sewers, in streams, and, in fact, under most
cial conditions. The second fact of importance is the difficulty
covering the typhoid organism under experimental conditions like
described.

thorough study of the bacteriology of sewage and of filter effluents
Houston to conclude that the biological processes at work in a filter
nk were not strongly inimical, if hostile at all, to the vitality of
ogenic germs.

A conservative study of all the evidence bearing upon this im tant question including the vitality and fate of certain non-pathog species, such as *B. coli*, leads to the conclusion that the remova pathogenic bacteria in purification methods is due to two allied cau the efficiency of which can be approximately determined. Ther first the time element and the known rapid decrease in the number certain bacteria such as *B. typhosus* when placed under conditions preclude multiplication. The rate of decrease varies but is rou about 50 per cent in twenty-four hours.

The second factor, acting in reality in conjunction with the f is the mechanical hindrance that is offered to the free passage of pended materials through the body of a filter. Even fine sand o little straining action as such, since the open channels are thous of times as big as the bacterial cell, but surface tension phenon tend to make all solid material adhere to the medium and thu passage is delayed. This action is prominent although of less im tance in coarse-grained filters. Actual experiments by the writer indicated that while the liquid may pass through a trickling in half an hour, small suspended particles such as ultramarine an *prodigiosus* cells require an average of over twenty-four hours. this way the actual time of passage is greatly delayed even when cc broken stone is the filter medium, and the times that are now kn to be necessary for the passage are ample in themselves to accoun the reductions that have been noted.

It may therefore be stated as a conservative view of the effici of purification processes in the removal of pathogenic bacteria, there are no strongly inimical processes at work in the tanks or fil and that the rate of decrease is not materially greater than woul observed in the same period of time under the conditions of a run stream.

The Cultivation of Sewage Bacteria

There are two general methods employed for the cultivatio those bacteria which are of assistance in sewage purification. may be cultivated in so-called filters of sand or coarser materia in specially constructed tanks such as the septic or the hydrolytic In the former case the bacterial growth occurs upon the special me provided, the sand or stone; in the latter, it takes place in the li

and a continuous life history within such a tank is possible only the rate of flow is sufficiently slow to permit of the inoculation of ncoming stream by the contents of the tank.

ILTERS.—The filtering media most commonly employed are sand rushed stone or other coarse material. In natural sand beds a

IG. 117.—Sewage Experiment Station, Mass. Inst. Technology. Trickling in front, sand filter just behind filter, dosing tank just behind sand filter, and tank just behind dosing tank.

period of treatment with sewage suffices to produce an active of "nitrification." By this term is indicated all the complex esses of oxidation one index of which is the formation of nitrates. such a filter has once become active in this way it will continue, proper care, to oxidize sewage almost indefinitely. Improper care, as an overdose of sewage or continued flooding of the surface due oor drainage, will soon destroy the activity of the filter. The addi- of germicidal substances has a similar effect and cold weather some-

what reduces the efficiency. From all this it is apparent that a filt is a biological culture medium upon which the various types of bacte are growing and carrying out their functions and that such a mediu requires careful control and is sensitive to unfavorable changes environment (Fig. 117).

The other filters are similar to this and illustrate the true function filtration. In the case of the sand filter it might be maintained th filtration or straining was an essential element in the process, but in t case of these coarse-grained media straining action is eliminated. He there is nothing but a pile of stones, varying from 1 to3 in ches more in diameter, upon the surface of which the bacteria grow. T

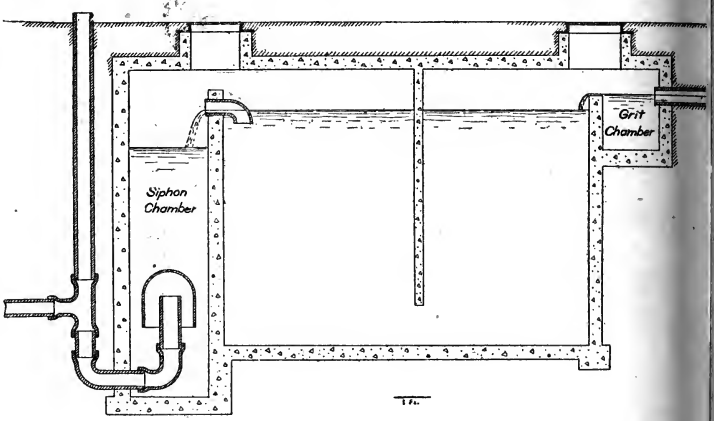


FIG. 118.—Sketch of septic tank. (Original.)

sewage trickles slowly over the surfaces, or is held in contact with th temporarily, according as we are dealing with trickling or contact filte Solids adhere to the stones or settle upon them, and soluble materia "absorbed" by the surface growth and removed from solution. Witl these gelatinous growths to which the air also has free access, the pr esses of oxidation take place and the products, the semi-oxidi organic material, are later "shed" from the stones appearing again the effluent as humus or stable organic matter.

ANAEROBIC TANKS.—The cultivation of bacteria in anaerobic tar is not quite as simple a matter as that which has just been describ

The sewage is allowed to flow slowly through the tank and after some time, from a few days to a month or more, a normal and constant flora will have become resident there. This flora will soon have become so well established that the incoming sewage laden with a flora of its own mingles with a liquid in which the established flora is so greatly in excess that the former in large measure gives way to the latter. In this way, while the sewage itself moves onward and is gone within a few hours, the flora is constant and persistent. A further aid in preserving this constant flora is the sludge at the bottom, in which the bacteria lodge and multiply and from which they are carried upward by the ever moving eddies and constantly re-inoculate the liquid above (Fig. 118).

The Destruction of Sewage Bacteria

By Biological Processes.—Reference has already been made to the effect of biological processes of purification upon pathogenic bacteria. What was stated in regard to the pathogens is equally true of the sewage bacteria as a whole. Their destruction is due to time and an environment unfavorable to growth, rather than to any specific cause. Further evidence of these facts may now be given. Bacteria as a whole do pass even the fine-grained filters in large numbers. Careful analyses of their types show them to be a haphazard mixture from the original sewage flora with little or no observable selection. Houston pointed out the relative abundance of the streptococci, supposedly delicate organisms, and found on the whole that the relative abundance of the different kinds of bacteria seemed to be much the same in the effluent as in the crude sewage.

On the whole we may conclude that the biological processes remove bacteria not by any specific antagonistic action but by delaying their passage and permitting the natural decrease that occurs when multiplication is prevented. The more efficient the mechanism of the filter in producing this delay the more complete will be the removal.

By Chemical Processes.—A much more reliable and economical method for bacterial destruction is now available in chemical disinfection of sewage effluents. The writer's studies at Boston, Baltimore and elsewhere have shown that the application of hypochlorite of calcium in amounts depending upon the character of the effluent, and

ranging from one to five parts per million of available chlorine (25 to 125 pounds of bleaching powder per million gallons), will produce a bacterial removal amounting to 98 or 99 per cent. This disinfectant is the most efficient of the known germicides, cost being considered. By this means it is possible to practically eliminate the bacteria, good and bad, from an effluent and it is no longer necessary nor desirable to seek high bacterial removals in the purification process proper. By thus dividing the work of purification into its component parts each part can be carried out at a maximum of efficiency and economy.

DIVISION III*

MICROBIOLOGY OF SOIL

CHAPTER I

MICROORGANISMS AS A FACTOR IN SOIL FERTILITY

INTRODUCTION

Rational views on soil fertility were first presented, in a systematic way, by Justus von Liebig in 1840. In his "Organic Chemistry in its Applications to Agriculture and Physiology" he developed important theories on the circulation of carbon and nitrogen in nature, and on the function of the so-called mineral constituents of plants.

When Liebig's book appeared, many of the leaders and students of agriculture still believed that humus, the partly decomposed residues of plants and animals in the soil, was the direct food of crops. They believed that soils could yield poor or rich harvests in proportion to the amount of humus present in them; they believed, in other words, that plants, like animals, used organic substances as food.

Liebig rendered a great service to agriculture in emphasizing the significance of decay processes. He made it evident that humus as such is of no use to plants, and that it becomes valuable only in so far as it is resolved into the simple compounds carbon dioxide, ammonia, nitric acid and various mineral salts. To be sure, he regarded the decomposition of organic matter as a phenomenon purely chemical, nevertheless he succeeded in showing that decay, putrefaction and fermentation are fundamental facts, connecting links between the world of the living and the world of the dead.

The research of the following decades brought to light the intimate relation existing between microörganisms and the decomposition of

*Prepared by Jacob G. Lipman with exception of sub-chapter on "*Soil Inoculation*" which has been prepared by S. F. Edwards.

organic matter. In the realm of soil fertility the new discoveries re vealed the vastness of the task assigned to soil microörganisms i providing available food for crops. It was shown that under the attac of bacteria and of other microörganisms the various organic débris i the soil is split into relatively small chemical fragments; that th carbon is restored to the air as carbon dioxide; that the nitrogen i changed into ammonia, nitrites and nitrates. It was shown, further that in this breaking down of organic matter the various cleavag products, and, particularly, carbon dioxide, hasten, to an amazin extent, the weathering of the rock particles and make available thereb the mineral portion of plant food. It was shown, likewise, that apar from accomplishing the *transformation* of unavailable into availabl plant food, microörganisms are concerned also in the addition c nitrogen compounds to the soil. The evidence gathered slowly b many investigators made it plain, therefore, that microbes are a important factor in the growing of cultivated and uncultivated plant Hence, the important place assigned to microörganisms in the study c soil fertility problems.

The Soil as a Culture Medium

Arable soils present so wide a range of conditions as to modif materially, the development and predominance of different specie Variations as to moisture, temperature, aeration, reaction, food suppl and biological relations are important, in each case, in determinin the survival or disappearance of any particular species. For th reason, the study of soil microörganisms must reckon with the mecha ical composition of soils, their ability to retain water and their conte of inert and soluble plant food.

Moisture Relations in the Soil

Amount and Distribution of Rainfall.—Precipitation different regions of the earth's surface varies from practically nothi to more than 1,524 cm. (600 inches) per annum. A portion of th water runs off the surface into the nearest stream, another portion rapidly changed into vapor and is returned to the atmosphere, and t remainder passes downward, into the soil and becomes the mediu in which plant food is dissolved. It is estimated that only about h

t total rainfall percolates through the soil. Where the soils are o n and nearly level the proportion of percolating water is relatively g ater; where the soils are fine-grained and more or less impervious, o the topography broken, the proportion is relatively smaller.

Bacteria and other microörganisms, as well as the higher plants, are d ectly influenced by the amount of moisture available for their various n ds. Hence soil microbial activities are affected not alone by the a ount of rainfall, but also by its distribution. It is obvious, for i tance, that an annual rainfall of 762 mm. (30 inches) distributed r her uniformly throughout the year would produce different soil-m isture relations than the same amount of precipitation confined to o y two or three months. As is pointed out by Abbe, a daily pre-c itation of 2 mm. (.079 inch) distributed throughout the three s mer months would be quickly changed into vapor, and would h dly wet the soil; whereas the total quantity of 180 mm. (7 inches) e nly divided into ten or twelve rains would penetrate the soil to a c siderable depth, and would furnish very favorable conditions for m robial development. In a similar manner it is pointed out by Hil-g d that Central Montana, and the region in the vicinity of the bay o San Francisco, have each a total precipitation of 610 mm. (24 inches). B t while in Montana the rainfall is distributed over the entire year a irrigation becomes necessary, the precipitation near San Francisco is imited to the portion of the year that nearly coincides with the g wing season, and crops are enabled to mature without irrigation.

Range of Soil Moisture.—Any given volume of dry soil consists o solid particles separated by empty spaces. The sum of these spaces is nown as the "pore-space." It varies from about one-third of the e ire volume in coarse sands to more than two-thirds in pipe clay. In p t and muck it may amount to as much as 80 or 90 per cent of the e ire volume. Under air-dry conditions each soil grain is surrounded b a very thin film of moisture designated as hygroscopic water. When a dry soil is moistened the films around the soil particles become th ker and finally cease to be isolated. A continuous liquid membrane, a t were, is stretched from particle to particle, and the surface tension th t thus comes into play is capable of lifting large amounts of water t the surface. The continuous film of soil water that can hold its o n against the pull of gravity is known as capillary water. Finally, w en the liquid films around the soil grains increase in thickness be-

yond a certain point, the attraction between the molecules in the s grains and the more distant molecules of water is no longer gre enough to overcome the force of gravitation, and the excess of wat percolates downward. The water more or less readily moved gravitation is called hydrostatic water.

For any given conditions of the soils the amount of hydrostat capillary and hygroscopic water is directly dependent on the mechani structure. It is evident that the aggregate surface of the particles a fine-grained soil is much greater than that in a coarse-grained so Actual determinations have shown that the aggregate inner surfa of .02832 c.m. (1 cu. ft.) of coarse sand may be but a fraction of acre; whereas the same quantity of the finest clay may have inner surface equivalent to 1.2141–1.6188 hectares (3 or 4 acre These differences are to be expected, since, as is shown by Lyon a Fippin, 1 g. of fine gravel may contain 252 particles; 1 g. of mediu sand, 13,500 particles; 1 g. of very fine sand, 1,687,000 particles; 1 of silt, 65,100,000 particles, and 1 g. of clay, 45,500,000,000 particles.

Since the soil water is spread as a film over the solid particles a varies in amount with the fineness or coarseness of the soil, and sin the quantity of plant food going into solution is determined large by the amount of water in contact with the soil particles, it follows th clay soils will, under the same conditions, contain more plant food solution than loam soils and still more than sandy soils. From t standpoint of soil microbiology this is important, for the microörganis live and multiply in the film water surrounding the soil particles. T concentration of salts in this film water as well as their compositi must of necessity affect bacterial activities. In the same way, metho of tillage and cropping affecting the concentration and compositi of the film water will modify the chemical changes caused by bacte and other microörganisms.

Effect of Drought and of Excessive Moisture.—Optimu conditions for plant growth and the development of many importa soil bacteria are furnished when about half of the entire pore space filled with water. In light sandy soils the optimum moisture conte may be reached when the wet material contains sarcely more than to 10 per cent of water by weight; while in silt and clay soils t optimum may reach 16 to 20 per cent. or even more.

Continued depletion of soil moisture by plant roots and evaporati

a the surface causes the film of capillary water to stretch more and
n re. Finally it becomes very thin, breaks, and ceases to be con-
t uous. The soil then becomes air-dry and contains only hyrgo-
s pic water. It is estimated by Lyon and Fippin that, under average
c ditions of humidity, light sand will contain 0.5 to 1 per cent of
h roscopic moisture; silt loam, 2 to 4 per cent; and clay, 8 to 12 per
c t. The amount of water present in air-dry muck or peat may range
u to 40 per cent, or even more. According to Hall the film of hygro-
s pic moisture is about 0.75μ (0.00003 inch) thick. As the soil
d es out bacterial activity is suspended and many vegetative cells
u loubtedly perish. Nevertheless, it will be seen that the moisture
fi even in air-dry material is deep enough to allow the bacteria a
r sonable degree of protection. This will account for the survival
o non-spore-bearing bacteria in dry soil for a long time. Indeed, in-
s nces are on record of the isolation of *Azotobacter* and *Nitrosomonas*
fr n soils that had been kept in a dry state in the laboratory for
s eral years. It may be noted, in this connection, that in the process
o drying the soluble salts in the soil the moisture may be sufficiently
c centrated in the films to cause plasmolysis and the destruction
o individual cells.

On the other hand, excessive moisture in the soil is not only directly
u avorable to aerobic species in that it limits their supply of oxygen,
b is objectionable because it encourages the formation of reduction
p ducts that are toxic to these species. It is apparent, therefore, that
f orable conditions for the formation of available plant food by
b teria are created when a certain relation is established between the
v umes of moisture and air in the soil. The shifting of this relation in
o direction or another is bound to react on species relationships and
n nbers.

Aeration

Mechanical Composition of Soils.—Soil ventilation is an impor-
t t factor in crop production. It provides for the proper supply of
e entary oxygen so essential to decomposition processes in normal
s s; for the supply of elementary nitrogen required by nitrogen-fixing
s cies; for the removal of excessive amounts of carbon dioxide; and
f the destruction of various toxic substances. The intimate relation

existing between soil ventilation and the mechanical composition of t soil material is bound to react on the microbial factors involved. It well known that the rate of flow of air through soils is inversely propo tional to the fineness of the material; in other words, the fine-grain soils, notwithstanding their greater pore space, will not allow air pass through them as rapidly as coarse-grained soils. King shows, f instance, that 5,000 c.c. of air passed through a column of fine gra in thirty-seven seconds, whereas in similar columns of medium san fine sand, loam and fine clay soil the same amount of air required for i passage 1,178, 44,310, 282,200, and 2,057,000 seconds respectively.

Aerobic and Anaerobic Activities.—The more rapid diffusi of gases from open soils naturally leads to a more frequent renewal their oxygen supply. In its turn, the latter affects the ratio of aerob to anerobes; it follows, therefore, that in clay soils and clay loam so the activities of aerobic species are retarded to a greater extent th they are in sandy loams or sandy soils. It follows, also, that in fin grained soils the activities of the aerobes are confined to a shallow soil layer than in coarser grained soils. The reverse is true of anaerob species. Methods of soil treatment tending to improve soil ventilati react both on the amount of chemical change produced by defini species, as well as the numerical ratio of different species to one anoth Among such methods may be included drainage, liming, manuring a tillage.

Rate of Oxidation of Carbon, Hydrogen and Nitrogen. Experiments carried out by Wollny proved conclusively that the pr duction of carbon dioxide in soils is directly affected by the amount oxygen supplied; that is, by the more or less thorough aeration of t soil. In one of these experiments air containing varying proportio of oxygen and nitrogen was passed through columns of soil. Wh this air contained 21 per cent of oxygen there were produced for eve 1,000 volumes of air 12.51 volumes of carbon dioxide; while with 2 p cent of oxygen in the entering air there were produced only 3. volumes of carbon dioxide. Similar observations were made l Schloesing in connection with the formation of carbon dioxide and nitric acid. Dehérain and many others have recorded the favorab influence of aeration on the rate of nitrate formation, while Lipm and Koch have observed an increased fixation of nitrogen by *Azotobact* consequent upon a better supply of oxygen.

THE MINERALIZATION OF ORGANIC MATTER.—Conditions that favor the intense activities of decay bacteria lead to a relatively rapid restoration of the phosphorus, sulphur, calcium, magnesium and potassium that had been made fast in plant tissues, to the stock of available plant food in the soil; indeed, in extremely well-aerated soils the decomposition of organic matter and its ultimate mineralization proceed too fast. It often happens that the farmer is unable to maintain a proper supply of humus in these soils because of their openness and is forced to adopt measures that will retard soil aeration. He resorts therefore, to rolling, marling, manuring and green manuring.

On the other hand, heavy, fine-grained soils are not sufficiently well aerated to allow a rapid mineralization of the organic matter. Under extreme conditions the decomposition processes do not keep pace with the process making toward the accumulation of organic matter, and a more or less considerable increase in the amount of the latter takes place. This occurs in low lying meadows, and, more particularly, in bogs and swamps. Hence the farmer attempts to intensify aeration and the resulting mineralization of the humus by more thorough tillage, drainage, liming and manuring.

TEMPERATURE

INFLUENCE OF CLIMATE AND SEASON.—An illustration of the differences that may exist in the soil temperatures of different regions is given by a comparison of the mean temperatures of 1901 recorded at Moscow, Idaho, and New Brunswick, New Jersey. The soil temperatures were taken to a depth of 152 mm. (6 inches).

SOIL TEMPERATURE,* 1901

	Jan.	Feb.	Mch.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Moscow, Idaho...	32.0	30.0	35.0	40.0	52.0	58.0	68.0	72.0	57.0	50.0	40.0	34.0
New Brunswick, N. J.	31.5	28.6	35.3	47.9	57.9	72.1	76.4	73.4	68.5	56.0	41.1	33.4

AIR TEMPERATURE,* 1901

Moscow, Idaho...	30.0	30.5	38.3	44.0	56.9	55.0	65.5	69.6	50.3	50.5	39.5	39.0
New Brunswick, N. J.	30.8	21.8	39.1	48.3	59.2	70.9	77.4	74.6	67.6	54.6	38.6	32.6

* Recorded in Fahrenheit scale.

It will be observed that in the months of November to March t soil temperatures in the two places were nearly the same. On t other hand, in April to October the average temperatures at Ne Brunswick were for soil 14.5° (58°F.) and for air 22.5° (72°F.), r spectively; and in July they were 20.0° (68°F.) and 24.5° (76.4°F respectively. It will also be observed that there is an unmistakab relation between the corresponding air and soil temperatures.

As a further illustration of the relation of climate to temperature comparison may be made of the average daily mean temperatures Bismarck, North Dakota, for the period 1873–1895, and at Key We Florida, for the period 1872–1895.

DAILY MEAN TEMPERATURES* (AIR)

	Jan.	Feb.	Mch.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	D
Bismarck, N. D...	4.5	9.5	22.6	42.1	54.2	63.8	69.5	67.5	57.0	43.8	25.9	1.
Key West, Fla....	69.7	71.4	72.7	76.1	79.4	82.5	83.9	83.9	82.5	78.5	74.2	7

It is obvious from the figures given here that, because of the i portant temperature variations of different soil regions, the mic biological activities must be profoundly modified. But apart from t climatic variations already indicated there are seasonal variations any particular locality that are of great moment for soil microbiologi activities. Such differences are demonstrated by the temperatu of 1898 and 1902, taken to a depth of 152 mm. (6 inches), at N Brunswick, N. J.

SOIL TEMPERATURES*

	Jan.	Feb.	Mch.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	D
New Brunswick, N. J. (1898) ...	33.2	33.1	45.1	48.9	59.1	76.0	79.3	77.8	72.0	60.1	44.6	3
New Brunswick, N. J. (1902)....	30.7	28.9	41.3	49.5	60.4	68.0	72.6	70.5	65.9	56.4	48.6	3

In this instance, the season of 1898 was not only earlier, but t temperatures of June to September were sufficiently higher to fa more intense bacterial growth and activity.

* Recorded in Fahrenheit scale.

EARLY AND LATE SOILS.—Under any given climatic conditions the warming up of soils in the spring will depend on their chemical and mechanical composition, color, tillage and topography. Because of the high specific heat of water, fine-grained soils containing a relatively large amount of moisture will warm up more slowly than coarse-grained soils containing a relatively small amount of moisture. The differences in the specific heat of humus, sand, clay and chalk are less important, yet they introduce appreciable variations in the soil temperature according to the proportion of each present. The topography of the soil introduces a factor of some importance for it affects the inclination toward the sun's rays as well as the drainage conditions. Tillage operations are of considerable moment, since they influence the rate of evaporation, that is, the rate at which heat is lost from the soil by the transformation of liquid water into vapor. Finally the color of soils exerts an influence on their temperature in that it affects the absorption and reflection of heat.

Taking all of the factors together, it is found that sandy soils and sandy loams are early soils, because they part readily with their excess of water. Clay soils and clay loams are, on the other hand, late soils; which means, therefore, that in the more open soils microbial activities become intense earlier in the spring. Market gardeners usually attempt to improve matters still further by the use of large quantities of readily fermentable manure that develops enough heat to raise slightly the soil temperature.

PRODUCTION AND ASSIMILATION OF PLANT FOOD.—It was already observed by Möller that slight amounts of carbon dioxide may be evolved from frozen soil. Kostychev could detect a considerable production of carbon dioxide at 0° to 5°. In a series of experiments carried out by Wollny the amounts of carbon dioxide produced were as follows:

CO_2 IN 1,000 VOLS. OF AIR

Water in soil	10°	20°	30°	40°	50°
5.79 per cent.....	2.03	3.22	6.86	14.69	25.17
5.79 per cent.....	18.38	54.22	63.50	80.06	81.52
5.79 per cent.....	35.07	61.49	82.12	91.86	97.48

The increased production of carbon dioxide at the higher temperatures, as shown in the above table, correspond with the observations that had

already been made by Ebermayer, Schloesing and others, that carbon dioxide production in the soil is greater in summer than it is in winter. These facts, taken together with the early observations of Forster on the multiplication of photo-bacteria at 0°, and the more recent observations of numerous investigators on the multiplication of individual species, or of mixtures of species in milk, water, soil, butter, etc., at 0°, or even below that, make it evident that bacterial activities are not entirely suspended at relatively low temperatures. As the latter rises these activities become more intense as gauged by the formation of carbon dioxide.

Coming down to specific groups of soil bacteria, it may be noted that at 12° nitrification is already quite perceptible; that urea bacteria grow slowly at 5°; *Ps. radicicola* at 4°; members of the *B. subtilis* group at 6° to 10°, etc. At 15° the breaking down of organic matter is fairly rapid, and at 25° the optimum is reached for many species. It follows thus, that the production of plant food—namely, ammonia, nitrates, sulphates, phosphates, etc.—gains rapid headway as the optimum temperatures are approached. The organic matter itself, apart from serving as a source of plant food, furnishes carbon dioxide and various organic acids that help to attack the rock fragments and to render available compounds of phosphorus, potassium, calcium and magnesium. It is likewise evident that in warm countries bacterial activities are not only more intense at any one time, but they continue through a longer period. For this reason, the soils of the South can furnish both relatively and absolutely a greater amount of available plant food than the soils of the North.

The production of plant food is necessarily followed by more vigorous growth of bacteria and of higher plants. More food is, therefore, assimilated and more moisture used up until the very rank growth of the crops hastens the depletion of the soil moisture. In this manner the soil may be dried out sufficiently to retard seriously the growth of soil bacteria and to retard thereby the decompositon of organic matter; under such conditions, moisture, rather than temperature, becomes the controlling factor of growth.

Reaction

Range of Soil Acidity.—Acid soils are very common in humid regions. The older soils of Europe include extensive areas whose lime

ontent has been restored repeatedly by the application of wood ashes, narl, oyster and clam shells, and various grades of burned or crushed imestone. In the United States acidity is becoming prevalent in many f the cultivated soils, as is shown by the investigations of the Rhode sland, Ohio, Illinois, Oregon and Florida experiment stations. These nvestigations, confirmed by experiments in other states, show that here is a marked removal of lime and of other basic materials from the oil as cultivation and the use of commercial fertilizers become more horough. Knisley shows, for instance, that 38.75 per cent of the regon soils examined were acid, and that 16.25 per cent were strongly cid. Similarly, Blair found that of 189 samples of different Florida oils and subsoils, examined, 68.22 per cent of the former and 51.35 er cent of the latter were acid. He also found that virgin soils were ss acid than cultivated soils.

Causes of Soil Acidity.—Soil acidity may be due to acids or acid alts, both inorganic and organic. Under ordinary conditions the tter are of much greater importance than the former as a cause of oil acidity. This is demonstrated by the extremely acid conditions f peat and muck soils that are particularly rich in organic acids. In oils left to themselves the formation of basic substances in the break- ng down of silicates and other compounds keeps pace with their eutralization by acid and their removal in the drainage water. When oils are placed under cultivation, lime and other bases are removed ore rapidly and the inert humic acids are left behind. The loss of ases is intensified by application of acid phosphate, potash salts and mmonium sulphate, commonly used as fertilizers. This accounts r the less extensive acidity in and among virgin soils as compared ith cultivated soils. Arid soils lose scarcely any of their basic sub- ances by leaching and are seldom acid. Residual limestone soils ay be alkaline, neutral or acid, according to the loss of bases they ave suffered by leaching. Low-lying soils, including meadows nd swamps may accumulate large amounts of organic acids because their imperfect aeration.

Effect of Reaction on Numbers and Species.—Some of the nportant groups of soil bacteria including nitro, azoto and ammonify- g species will develop slowly or not at all, when the amount of acid in e medium is increased beyond a certain point. Hence it is realized y progressive farmers that a proper supply of lime is essential for the

satisfactory decomposition of organic matter in the soil, and the abundant supply of available nitrogen compounds, as well as of other constituents of plant food to growing crops. The influence of lime on the multiplication of soil bacteria is well illustrated, for instance, by the experiments of Fabricius and von Feilitzen. These investigators found only 138,500 bacteria per g. in newly broken and unlimed peat soils; whereas in similar soils that had been limied and cultivated for several years the numbers averaged about 7,000,000 per g. and reached a maximum of 22,132,000 per g.

Food Supply

Organic Matter.—It may be said truly that a soil devoid of organic matter is practically devoid of bacteria. To the fresh and the partially decomposed organic matter (humus) the soil organisms must look for most of their food and energy. Being largely of plant origin this organic matter contains starches, fats, organic acids, higher alcohols, proteins and amino-compounds. Because of the different relations that these vegetable substances bear to the several species of soil bacteria, a high or low proportion of starch, of cellulose, or protein must necessarily modify both numbers and species relationships. For instance, observations have been made by Coleman and others that small amounts of dextrose favor nitrification, whereas larger quantities retard it; similarly, it has been noted that in the spontaneous decomposition of protein bodies bacteria are prominent and molds absent or relatively few in numbers. But where dextrose is added to the decomposing proteins molds soon appear in large numbers. There may also be cited, in this connection, the observation of Hilgard that humus should contain at least 4 per cent of nitrogen if it is to furnish a sufficient quantity of available nitrogen compounds; otherwise, the soil bacteria seem to be unable to decompose it, so as to meet the needs of the growing plants. Many similar facts could be cited to show that as a culture medium the soil is influenced by green manures, barnyard manure, commercial fertilizers, lime, tillage and any other treatment that will modify the quantity as well as the quality of its organic matter.

The Mineral Portion of the Soil.—The moisture films surrounding the soil grains contain in solution substances derived from

ese soil grains. A particle of calcium carbonate will be surrounded y a moisture film containing some calcium bicarbonate. In the me way particles of feldspar may give rise to a solution of potassium icarbonate; particles of apatite to a solution of calcium phosphate; articles of selenite to a solution of calcium sulphate; particles of rotein to a solution of ammonia, etc. In view of the fact that these actions are more or less localized and diffusion slow, there are, unoubtedly, in the soil minute zones where individual species are more rominent than they are in others. For example, Heinze has found it onvenient to isolate *Azotobacter* by inoculating suitable culture solutions with particles of calcium carbonate picked out from the soil. vidently these organisms were present in much greater abundance n these particles than on others of non-calcareous origin. Indeed, e occasionally obtained in this manner *Azotobacter* membranes that onstituted almost pure cultures. The more general significance of is relation is apparent when it is remembered that nitro-bacteria e particularly favored by magnesium carbonate; tubercle bacteria y gypsum and calcium carbonate; *Azotobacter* by calcium phosphate nd calcium carbonate; photo-bacteria by sodium chloride, etc.

Considerable as must be the local differences in any one soil, they e undoubtedly even more pronounced when different soils are compared. Extreme conditions are met with in certain irrigated soils which a marked concentration of salts occurs. In so far as crop oduction is concerned, it is stated by Hilgard that the upper limit is actically reached when the concentration of soluble salts in the irrigaon water is about 4.55 g. (70 gr.) per gallon. Nevertheless, in Egypt nd the Sahara region irrigation water is occasionally used that contains more than 13 g. (200 gr.) of soluble salts per gallon. Further fferences are introduced by the quality of these salts, *e.g.*, the proortion of sodium sulphate, magnesium sulphate, sodium chloride, dium carbonate, etc. Again, instances are on record, as in the investitions of Headden in Colorado and California, where the concentration nitrates in the soil water is so great as to kill even relatively resistant ants like alfalfa. It is to be shown by future investigations what the fect of the concentration and composition of such salts may be on the il bacteria.

In humid soils conditions are less extreme, yet even here the variable ncentration and composition of the soil solution are of direct moment

for the different microörganisms. Granite soils, for instance, are fairly well supplied with phosphoric acid and abundantly with potash, but when hornblende is lacking they are apt to be deficient in lime. Ill-ventilated clay soils may contain reduction products of iron salts, while green sand, chalk, slate, shale, sandstone and other soils may have their individual peculiarities from the standpoint of a culture medium.

Biological Factors

Molds.—*Distribution.*—While the study of the lower bacteria in the soil has attracted the attention of many investigators, that of fungi and actinomyces received until recently, but scant consideration. Fungi occur in all soils, cultivated as well as uncultivated, rich or poor in organic matter, heavy or light in texture. Most of them are obligate saprophytes, although facultative parasites are found in large numbers in the soil, especially where single-cropping or short rotations favor the survival of the particular organisms. The isolation of soil fungi has been accomplished either by the dilution method, where a sample of soil was shaken with water, and only a certain dilution was used for inoculation; and by the direct method, where a clump of soil was inoculated into a sterile medium, and the fungi developing on it were isolated. About 150 different species of fungi have been isolated from different soils, and the data accumulated by investigators in this country and in Europe seem to point to the fact that many of these fungi are universal in their habitat, since the same species are recorded to have been isolated from different soil types and in different localities. Most of the work done refers to the classification of the organisms isolated. The largest group of soil fungi belong to the following genera: *Mucor*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Trichoderma*, *Cephalosporium*, *Alternaria*, *Zygorrhynchus*, *Monilia*, *Rhizopus*, and *Acrostagmus*. Many other genera have been isolated, but to a more limited extent. As to the individual species occurring in the soil, Hagem, having isolated about 30 mucors from the soil, states that certain *Aspergilli* occur in larger numbers than all the mucors taken together. As to quantitative relations, no exact data are available. Some investigators report only several hundred fungi per g. of soil, while others record as many as 1,000,000 per g. of soil; that is the total number of spores and pieces of mycelium that develop on suitable

ıedia. As to the numbers and types in relation to depth, Goddard ɔncluded that there does not seem to be any appreciable variation in ıumbers at the different soil depths. Unpublished data of the New ersey Experiment Station bring out the fact that there are very few ıngi in the soil below 8 inches, and that one of the most common forms t these depths is *Zygorrhynchus vuilleminii*. It was formerly thought ıat soil fungi are abundant only in acid soils, but recent investigations ıake it appear that also limed and well-cultivated soils have an ɔundant fungus flora.

Ammonification.—Müntz and Coudon, and after them Marchal, ɔrking with pure cultures, proved conclusively that fungi decompose rganic matter and cause an accumulation of ammonia in the soil. Vilson and McLean found that the forms of *Monilia* are the most active mmonifiers among the several groups of organisms studied, while the *spergilli* showed the least ammonifying power. More recent work as confirmed the earlier findings and has proved that fungi may lay an active part in the decomposition of organic matter, and the ccumulation of ammonia.

Nitrogen-fixation.—Experiments on nitrogen-fixation by fungi were arried on by Jodin as early as 1862. He observed a rich fungus growth n nitrogen-free media, supplied with sugar, tartaric acid, or glycerin. erthelot, Saida, Ternetz, and others also reported fixation of atmosheric nitrogen through the activities of fungi, such as *Aspergillus iger*, *Alternaria tenuis* and several species of *Monilia*, *Penicillium*, *Mucorini* and others. But other investigators among them, Winoradsky, Czapek and Heinze were unable to confirm these observaons. The careful work of Goddard has also given negative results. he entire question is therefore still an open one with the weight of vidence on the negative side.

Cellulose Decomposition.—The destruction of cellulose in the soil is ue to a large extent, to the activities of soil fungi, as has been demonrated by several investigators. Cellulose decomposition by fungi was rst observed in the study of plant diseases. Van Iterson used filter aper for the isolation of fungi, by exposing this medium to the air for welve hours. Thirty-five species of fungi were isolated thus proving hat a large number of cellulose-destroying fungi may be present in he air. Appel found that certain species of *Fusarium* destroyed in ɔurteen days 80 per cent of the filter paper used. Marshall Ward

and others recorded that a number of fungi are economically important as wood-destroyers. Spores of a pure culture of *Penicillium* sown on sterile blocks of spruce wood, germinated and grew normally. Sections of the wood showed that the hyphæ had entered the starch-bearing cells of the medullary rays of the sapwood and consumed the whole of the starch. MacBeth and Scales found that when the medium is slightly alkaline, certain aerobic bacteria will play the principal rôle in the destruction of cellulose. When the medium is acid, molds and higher fungi become the active agents of destruction. They also found that the cellulose-destroying forms multiply with great rapidity in alkaline soils when cellulose in the form of filter paper is added. The power to destroy cellulose is reported for a number of species of *Penicillium*, *Aspergilli*, *Trichodermæ* and other organisms which belong to the common soil forms. Though the fungi may play an important part as cellulose destroyers also in alkaline soils, in acid soils where the activity of bacteria is greatly inhibited, fungi probably play a predominant rôle. This fact led Marshall to conclude in 1893 that fungi take an active part in the mineralization of the organic matter in acid humus soils.

Mycorrhiza.—Apart from the so-called soil fungi, there exists another group known as mycorrhizal fungi. These live symbiotically on the roots of the higher plants. Many roots of forest trees, when examined carefully, show that there is a union between the mycelium of certain fungi, usually belonging to the fleshy fungi, and the root of the plant. This union is called a "*mycorrhiza*." The fine filaments of the fungus enter the cells of the root. These organisms were thought at first to supply the roots with water and soluble plant food from the soil. The power to fix atmospheric nitrogen has been ascribed to these organisms by several investigators. But aside from these useful so-called endotrophic *Mycorrhizæ*, there are also the ectotrophic *Mycorrhizæ* which probably live only parasitically upon the roots of plants.

Actinomyces.—The study of soil *Actinomyces* is nearly all of very recent origin. Several years ago but two soil *Actinomyces* had been definitely described, viz., *Act. albus* and *Act. chromogenus*. The work of Krainsky, of Conn and of Waksman and Curtis has demonstrated that *Actinomyces* are widely scattered in cultivated soils. The last named investigators have shown that while the absolute numbers of *Actinomyces* decrease with depth of soil, their relative numbers are

nterially increased so that if at a depth of 25 mm. (1 inch) there a only 6 to 10 per cent of *Actinomyces* and 82 to 93 per cent of bcteria, at a depth of 750 mm. (30 inches) the *Actimonyces* form 4 to 80 per cent of the total micro-örganic flora of the soil. The mbers of *Actinomyces* in the surface soil vary greatly with the types soil and abundance of plant food. In one instance 1,300,000 *Actinnyces* were found in a total of 15,000,000 bacteria per g. of rich adow soil. As to the activities of *Actinomyces* in the soil, Beyerik has shown that the *Act. chromogenus* produces an oxidizing subsnce, quinon ($C_6H_4O_2$) which may play an important part in the odation of organic matter in the soil. Munter, Krainsky and Scales ve demonstrated that many *Actinomyces* are able to decompose cellule in the soil, and that in some instances this ability is very marked. ainsky records that soil *Actinomyces* need very little nitrogen for ir life activities, and that they can get it from any available source. nitrates are present, these are reduced first to nitrites, and then ulized. Waksman and Curtis working with soil sterilized by steam, d not find any great accumulation of ammonia through the activities o*Actinomyces*, although different species seemed to show marked variatn in their power to accumulate ammonia.

Algæ.—At times the influence of algæ in changing the character of t soil as a culture medium for bacteria is quite considerable. As corophyl-bearing organisms they are enabled to manufacture sugar a l starch with the aid of sunlight, and to favor thus the development o *Azotobacter* and of other microörganisms dependent for their energy o the organic matter in the soil. Investigators both in France and iGermany have found that the fixation of nitrogen in sand used for p culture experiments occurs in the surface layer possessing a growth o algæ. The advocates of bare fallows attribute the greater prodctivity of fallowed land to the growth of algæ, the accumulation of nrogen through their influence and to other changes affecting the soil bteria.

Protozoa.—It has been shown for a long time that certain species o protozoa are common in soils and that their food consists of bacteria. T what extent protozoa play a part in soil fertility has not yet been fy explained, even though Russell and Hutchinson and of the Rthamsted Experiment Station have maintained that these minute a mals are extremely important in that they maintain a certain bac-

terial equilibrium in the soil. Their claim is mainly based on the fac that partially sterilized soils (either by means of heat or antiseptics soon come to contain enormous numbers of bacteria.

It is, therefore, assumed by them that this abnormal increase i made possible by the destruction of the protozoa (which have a lowe power of resistance to heat and antiseptics than bacteria) that normall check the increase beyond a certain point. Under the conditions re corded a causal relationship obtains between an increase in numbers c bacteria and the rate of ammonia production, which is considered to b an index of fertility.

This theory has been the basis of considerable investigation, muc of which has failed to corroborate the above conclusions. The fac that there is an increase in bacterial numbers and in consequenc enhanced fertility of the soil may not be due to the elimination i protozoa but may rather be ascribed to such effects of the parti sterilization process as (1) increase in available food for bacteri (2) rendering soil toxins insoluble; (3) destroying bacterio-toxin (4) acceleration of the biological processes.

It has even been noted in some instances that partial sterilizatic has been responsible for a decrease rather than increase in the produ tion of ammonia. Such considerations, among others, have been i strumental in stimulating investigation in another branch of soil fertilit namely, soil protozoölogy. There has been difficulty in establishi suitable methods and technic, as for example the development media favorable for isolation and the culture of soil protozoa; althou blood meal solution, hay infusion and soil extract have been used advantage. The organisms have been counted in the same manner bacteria, namely, by the dilution method, or by means of a standa platinum loop. An adaptation of the apparatus used in the counti of blood corpuscles has been successfully employed by Kopelo Lint and Coleman.

A study of the morphology and life history of soil protozoa reve the fact that encystment occurs under most conditions which are n immediately favorable, as for example slight variations in moistu content, or food. In point of fact this period of the protozoan life cy which is analogous to the spore-forming stage of bacteria forms t basis for the question which arises as to the existence of protozoa, their trophic form, in field soils. Of the well-defined groups of p

toa (page 130), namely, flagellates, ciliates and amœbæ, many types hae been described. Among those occurring frequently are: *Colpoda cullus, Boda ovatus, Colpidium colpoda, Amœbæ terricola, Monas*, etc. Te requirements for maximum development in the soil for these organisms are: (1) A high degree of moisture, closely approximating saturation; (2) an abundant supply of organic matter; (3) moderate temperature. Te thermal death point of active forms has been found by Goodey toe 40° to 50°, and for the cyst forms of the same organisms about 7 The optimum temperature for most forms is about 22°. Eystment of protozoa occurs within wide limits in an alkaline medium cotaining up to .18 per cent NaOH, and in the presence of an acidity reesented by .09 per cent HCl.

Protozoa are found in many greenhouse soils, due no doubt to the fa that they contain a high degree of moisture and organic matter. Hvever, in dealing with field soils some investigators have failed to isate active forms of protozoa, whereas others record the presence of la numbers of these organisms. Their distribution appears to pallel that of bacteria, namely, the greatest number of protozoa occurs wain the upper 100 mm. (4 inches) of soil, with a decrease down to 30 mm. (12 inches), which represents the lower limit of their activity.

As regards the occurrence of the various groups of soil protozoa, flellates are found to be dominant over ciliates and amœbæ. G. P. Kh has found that the development of soil protozoa in artificial cuure solutions varies (1) with the kind of media employed; (2) the qntity of soil used for inoculation; (3) drying of the soil; (4) different kils of soil and different soils of the same kind; (5) the temperature ofncubation.

While it is generally accepted that protozoa feed upon bacteria, uil the relation that obtains between the various types of protozoa ar the different species of soil bacteria has been more fully investigated th direct effect of protozoa upon bacteria must remain, to a degree, interminate.

Soil sterilization has had a practical application in eliminating vious diseases in greenhouses and infested fields. Partial sterilizatin as employed by Russell and Hutchinson while not so drastic, inolves serious changes in the soil, which might be considered in much th same light as the phenomena attending complete sterilization by means of heat and antiseptics. It is an established fact that

sterilization is responsible for increased plant growth, and to expla this phenomenon the following theories have been advanced:

1. R. Koch's theory of direct stimulation to plant growth—physiological effect of the sterilizing agency.

2. Hiltner and Störmer's theory of indirect stimulation—an alter tion of the bacteriological equilibrium resulting in a marked develo ment of numbers after decimation.

3. Liebscher's view that soil sterilization may be regarded in t same light as a nitrogenous fertilizer.

4. Russell and Hutchinson's protozoan theory of soil fertility.

5. Pickering and Schreiner's contention that the alteration chemical composition is largely responsible for increased plant growt

6. Greig-Smith and others adhering to the bacterio-toxin hypothes

Higher Plants.—Higher plants modify the soil as a cultu medium for bacteria in at least three ways. The root-hairs cor into contact with the moisture films surrounding the soil grains a not only modify the composition of the film water, by withdrawing portion of the dissolved matter, but also change its character by sec tions from the roots. The changes thus effected must, necessari modify the character of the soil and the soil solution as a cultu medium. Again, the rapid removal of water from the soil by growi crops causes the film water to become more concentrated in so far, least, as some salts are concerned. Modifications, are, also, introduc thereby in the proportions of oxygen, nitrogen and carbon dioxide the soil air. Finally, higher plants modify the soil environment bacteria by their root and stubble residues. For example, residues leguminous plants, being richer in nitrogen and possessing a narrow carbon-nitrogen ratio than the corresponding residues of non-legum will affect the soil somewhat differently than the latter.

Bacteria.—Occupying, as they do, the leading rôle, bacte demand a more detailed consideration, in fact, most of the biologi discussions of soil are based upon a knowledge of these organisms.

Numbers and Distribution (Bacteria in Productive and Unproduct Soils).—The numbers of bacteria in soils well supplied with orga matter usually range from 3,000,000 to 15,000,000 per g., as sho by the agar plate method. These numbers vary from soil to soil, a from season to season for any particular soil. The numbers of fu are also variable and may reach a total of 1,000,000 per g., althou

it still remains to be demonstrated whether the large numbers thus fo nd represent organisms which lead an active life in the soil or only sp res of fungi brought in by external agencies. The numbers of *Actinomyces* may reach 1,000,000 or more per g. of soil. The fungi a most disappear below 20 to 30 cm., while the actinomyces do not d crease rapidly at depths lower than 30 cm.

Distribution at Different Depths.—Most of the soil bacteria are found in the stratum in which the organic residues are concentrated, that is, in the surface soil. Immediately at the surface the rapid evaporation a l the germicidal effect of direct sunshine act as disturbing factors, h ce the numbers in the uppermost 25 to 50 mm. (1 to 2 inches) are s aller than in the layer of soil immediately below. Beyond the d th of 20 cm. or 22 cm. (8 or 9 inches) the numbers diminish rapidly. M terial from a depth of .6 m. to .9 m. (2 to 3 feet) is nearly sterile in h nid regions. Differences occur, however, in keeping with the m chanical composition of the soil. In light, open soils the bacteria a not only carried down to greater depths by the percolating water, b can also multiply there, thanks to better aeration. On the con- tr y, fine-grained compact soils are more effective in holding back s pended material and do not allow the bacteria to pass downward as r dily. Moreover, the less thorough aeration of these soils and the a umulation of toxic reduction products in the subsoil serve as an e ctive check in the increase of bacteria in the deeper layers.

In irrigated soils of the arid and semi-arid regions bacteria are dis- t uted at much greater depths. Their occurrence 2 m. to 3 m. (6 r 10 feet) below the surface is made possible not only by the better a ation of these soils, but by the penetration of roots to great depths a l the accumulation there of considerable amounts of organic matter. T e practical significance of distribution appears, among other things, in he use of soil for inoculation purposes; for instance, it is reported by S ström that in making peat soils arable the addition of small amounts o fertile loam increases to a very marked extent their crop-producing p wer. The efficiency of the inoculating material decreases as it is t en from the deeper soil layers. Similarly, in the use of alfalfa soil f the inoculation of new fields the most efficient material is secured at a epth between 7.62 cm. and 17.78 cm. (3 and 7 inches).

Seasonal Variations of Bacterial Numbers and Activities.—Conn has r orted an apparent increase of bacteria in frozen soil. This increase

seems to be due to an actual multiplication of the organisms rather tha to a mere lifting of the bacteria from lower depths by capillary actio The greatest increase was found to occur during the winter in the slo growing bacteria and not in those that liquefy gelatin rapidly or in t *Actinomyces*. Conn tries to account for the phenomenon by assumi the existence of two groups of bacteria, winter and summer bacter The latter, he thinks, prevents the former from multiplying rapid in warm weather. Hence, the increase in frozen soils is not to ascribed directly to the low temperature, but to the depressing eff of the cold upon the summer bacteria. Brown found that the s bacteria diminish during the fall season with the lowering of t temperature, but, when the soil is frozen, an increase in numb occurs. He also found frozen soils to possess a much greater ammo fying, denitrifying and nitrogen-fixing power than non-frozen so According to him, the lowering of the freezing-point of the capill water, due in part to the concentration of salts at the time of freezi may account for the abnormal bacterial activities.

Morphological and Physiological Groups (*Morphological Groups*) Rod-shaped organisms are numerically the most prominent among s bacteria. They occur at times to the extent of 80 or 90 per cent the total number. Spherical organisms usually constitute less th 25 per cent of the bacterial flora. Spirilla and sarcinæ are present slight numbers. Conditions may occur, however, when the proport of spherical organisms is markedly increased. This happens, p ticularly, when large quantities of composted manure (rich in spheri organisms) is added to the soil.

Among the rod-shaped species *B. mycoides*, *B. subtilis*, *B. mes tericus*, *B. tumescens* and other members of the subtilis group are q prominent. Members of the amylobacter group are seldom abse Members of the proteus group and various fluorescens are alw present, while *Bact. ærogenes* and allied species are common inhabita of the soil.

(*Physiological Groups*).—In the decomposition of organic matte the soil certain important changes in both nitrogenous and non-ni genous material are accomplished by definite groups of bacteria. breaking down of protein substances is accomplished by the for tion of ammonia, nitrites and nitrates. These in turn may be tra formed back into more complex amino-compounds, peptones, and

ins, or they may be destroyed with the evolution of free nitrogen. oreover, there are groups of bacteria capable of joining non-nitro- nous organic matter to elementary nitrogen and of producing thus trogen compounds. Again, there are groups of bacteria bearing stinct and important relations to the decomposition of cellulose, or e transformation of its cleavage products, methane and hydrogen. here are, likewise, definite groups of bacteria concerned in the ansformation of sulphur and its compounds, and of ferrous compounds.

Methods of Study

Quantitative Relations.—Since the early work of Koch in 1881 any investigators have determined the number of bacteria in soil mples, by means of the plate method. It is well known, however, at on ordinary gelatin or agar plates kept under aerobic conditions t a fraction of the soil organisms produce visible colonies. The aerobic species do not appear, nor do aerobic *Azotobacter*, and nitro- cteria, while other common soil organisms form colonies sparingly not at all. By employing synthetic agar media instead of beef broth latin or agar, Lipman and Brown have succeeded in securing the owth of a much larger number of colonies from any given quantity soil, yet even these larger numbers were incomplete for reasons entioned above.

H. Fischer recommends a simple medium of agar to which nothing s been added but soil extract (prepared by extracting with a .1 r cent solution of Na_2CO_3) and potassium phosphate. Following e path of Lipman and Brown in reducing the content of organic atter, Temple employed 1 g. of peptone per l. as a culture medium d obtained satisfactory results. Brown has further modified the rmula of Lipman and Brown by replacing the .05 g. of peptone with g. of albumin, and obtained results which were somewhat superior. a comparison of culture media, Conn considers the former media desirable for quantitative purposes because they contain substances indefinite chemical composition, and offers an agar medium con- ining no organic matter except agar, dextrose and sodium asparag- ate, and also a soil-extract gelatin which is valuable for qualitative rposes. Another medium that has been suggested, after a com- rison of all of the above-mentioned media, is the urea-ammonium

nitrate agar of R. C. Cook. It is evident, therefore, that the result secured in the counting of soil bacteria have only a relative value With the same media and methods some information may be secured concerning the influence of fertilization, tillage, liming, etc., on certain of the soil bacteria. But even this information must be properly discounted, since equal numbers do not necessarily mean equal amounts of chemical work accomplished; for example, there is no certainty that 1,000,000 of decay bacteria derived from one soil will accomplish exactly as much decomposition as the same number of similar organisms from another soil. Otherwise stated, individual cells differ in their *physiological efficiency* from other cells of the same species.

Qualitative Reactions.—By modifying the composition of the culture media different physiological groups may be favored in their development. In this manner the silica jelly medium proposed by Winogradski, or the gypsum plates proposed by Omelianski may be employed for making numerical comparisons of nitro-bacteria in different soils. In like manner Beijerinck's mannit agar may be used for the numerical comparison of *Azotobacter*, and other media could be adapted for the quantitative-qualitative determination of urea, denitrifying, methane, and still other physiological groups of microörganisms.

There is no doubt that the quantitative-qualitative method just outlined may be made to yield valuable information. Yet it, too, possesses defects already noted in connection with the more strictly quantitative method. Apart from the vast amount of work involved in the preparation of a large number of media and in the counting of colonies on many plates, this method fails to indicate differences in physiological efficiency. Furthermore, the colonies of the specific organisms sought are almost invariably accompanied by those of foreign species not always easily distinguished. With these limitations properly recognized and with further improvement in the constitution of special media the method may be made useful in supplementing data secured by other methods.

Transformation Reactions.—Instead of counting soil bacteria in accordance with colonies produced in general or special media, some investigators have attempted to measure the bacteriological functions of soils by comparing more or less definite quantities of the latter under known conditions. This method was employed by Wollny and others in studying the factors that affect the formation of carbon dioxide in

sls. It was also used by Schloesing and Müntz and their followers in similar studies on nitrate formation. A method somewhat similar in principle but different in its application was proposed by Remy in 1902. He suggested the use of special media for the quantitative determination of different physiological reactions; thus, making a 1 per cent solution of peptone and inoculating with equivalent quantities of soil, he caused the decomposition of the peptone and the formation of ammonia, and secured comparisons of the ammonifying power of different soils. In a similar manner he used special solutions for comparing quantitatively the transformation accomplished by nitrifying, denitrifying or nitrogen-fixing bacteria.

Remy's method has been extensively tested by Löhnis, Ehrenberg, Lipman and others. It has been shown to possess a serious defect in that it deals with conditions unlike those occurring in the soil itself. For this reason more recent investigations have been carried on in weighed portions of soil rather than in culture solutions inoculated with 1 per cent of soil as is done in Remy's method.

Rate of Oxidation of Carbon.—The rate of decomposition of humus or of other organic matter in the soil may be measured, as was done by Wollny, by determining the amount of carbon dioxide evolved in weighed quantities of material kept under definite conditions. The influence of temperature, moisture, aeration, organic matter, antiseptics, etc., has been determined in this manner. The same method may be used in studying decay, and factors influencing decay, in soils in the field.

More recently Russell and his associates have modified the method in that they have determined the rate of oxidation of carbon not by measuring the carbon dioxide evolved, but by estimating the amount of oxygen absorbed. In either case decay is measured from the carbon standpoint. The method based on this principle should find wide application in future soil fertility investigations.

Rate of Oxidation of Nitrogen.—Another method or series of methods for studying decomposition processes in the soil may be based on the determination of nitrogen compounds formed in the breaking down of proteins. Two of the derivatives of protein, namely, ammonia and nitrate, have been used successfully to gauge the decomposition of organic matter in the soil. The recent results secured by Lipman and his associates demonstrate that ammonia formation from dried blood in

weighed quantities of soil may serve as a very accurate measure decay from the nitrogen standpoint. Corresponding determination nitrates may similarly be employed in tracing protein cleavage ar transformation as influenced by the various factors of season, s and cultivation.

ADDITION OF NITROGEN.—At least one other bacteriological fact in soils should be mentioned here as deserving attention in a systemat study of soil fertility from the nitrogen standpoint. It is known th *Azo-bacteria* are widely distributed in arable soils, and that they a more prominent in some regions than they are in others. The stude of soil fertility finds it desirable, therefore, to study azotofication different soils, and employs (for this purpose) mannit solutions li those proposed by Beyerinck, sand cultures supplied with sugar sol tions like those proposed by Fischer, or weighed quantities of soil mix with sugar as suggested by Koch.

The methods referred to above make possible thus the study ammonification, nitrification and azotofication under controlled co ditions and permit, thereby, the measure of bacteriological factors soil fertility from the nitrogen standpoint.

REACTIONS CONCERNING CALCIUM, MAGNESIUM, SULPHUR A PHOSPHORUS.—In addition to the purely chemical methods available the study of these constituents, microbiological methods have also be suggested. In some of his still unpublished experiments with *Azo bacter* Lipman employed solutions of mannit in distilled water, provid with small quantities of sterile soils which were to supply the organis with the essential mineral constituents. In this manner interesti data were secured on the availability of phosphorus compounds different soils; similarly, Christensen has suggested the use of *Azo bacter* for determining the lime requirements of soils, and Butkev has experimented with cultures of *Aspergillus niger* in determining availability of the mineral constituents.

CHAPTER II

HE DECOMPOSITION OF ORGANIC MATTER IN THE SOIL

Carbohydrates

Origin.—The sugars, starches, vegetable gums and allied pectine ıbstances, as well as the cellulose, contained in roots and other crop sidues add large quantities of carbohydrates to the soil. The crop sidues are augmented still further by green manures and animal anures whenever these are used. A good growth of timothy, for xample, may increase the content of organic matter in the surface il by 250–500 kg. (500 or 1,000 pounds) per acre, and three-quarters this consists of carbohyrdates. In the same manner, a green ma-ure crop, or an application of barnyard manure may add to the land much as 1,500 pounds, or even more, of carbohydrates per acre. hese carbohydrates contain a large proportion of cellulose.

The Decomposition of Cellulose.—Pure cellulose (page 167), $C_6H_{10}O_5)_x$ is a rather inert substance, as exemplified by the resistance cotton and flax fiber to decomposition processes. It is well known, evertheless, that even cellulose is in the end decomposed and resolved to simple compounds. Plant roots, leaves and stems, as well as the unks of fallen trees, gradually disintegrate and vanish. Under favor-ble conditions this may proceed rapidly, as is indicated by the process septic tanks, or in manure heaps on the one hand, and in open ndy soils on the other. The disappearance of cellulose may be ac-mplished by (*a*) anaerobic organisms, (*b*) by aerobic organisms, (*c*) y denitrifying bacteria, and (*d*) by molds.

The Production of Methane and Hydrogen.—The decomposition pure cellulose and the formation of methane and hydrogen mixed ith other gases was first noted by Popov in 1875. Some years ter Tappeiner and also Hoppe-Seyler confirmed Popov's observa-ons that nearly pure cellulose in the form of Swedish filter-paper, or tton fiber may be fermented by bacteria with the evolution of ethane, carbon dioxide and occasionally also of hydrogen. These

investigators ascribed the decomposition of cellulose to an organism found by Trécul in decomposing vegetable materials, and named by him *Amylobacter* in 1865, because of the blue color assumed by it when stained with iodine.

Subsequent investigations by Omelianski begun in 1894 and continued through a period of years demonstrated the presence of specific anaerobic organisms in decomposing cellulose. He described two distinct species of long, slender bacilli, assuming the clostridium form when in the spore stage. Morphologically the organisms can hardly be distinguished, but physiologically they show important differences in that one causes the fermentation of cellulose with the production of gases consisting of carbon dioxide and methane, while the gases produced by the other consist of carbon dioxide and hydrogen; hence the one is designated by Omelianski as the methane bacillus and the other the hydrogen bacillus. These organisms do not stain blue with iodine, and do not belong, therefore, to the butyric bacilli designated as *Amylobacter* by earlier investigators. Omelianski's investigations make it appear that the butyric organisms are not capable of fermenting cellulose proper.

In culture solutions containing mineral salts and nitrogen in the form of ammonium compounds the decomposition of filter-paper and other forms of cellulose proceeds with considerable rapidity. Calcium carbonate must be added to neutralize the acids formed, otherwise the fermentation soon comes to a standstill. In one of Omelianski's experiments begun in October, 1895, and ended in November, 1896, 3.347 g. of cellulose was decomposed by a nearly pure culture of hydrogen bacilli. The products consisted of 2.2402 g. fatty acids, .9722 g. carbon dioxide and .0138 g. of hydrogen, a total of 3.2262 g. which nearly accounts for all of the cellulose destroyed. The fatty acids were made up of butyric and acetic acids with a slight proportion of some higher homologue, probably valerianic acid.

In a similiar experiment with an apparently pure culture of the methane bacillus, begun in December, 1900, and ended in April, 1901, fermentation began after an incubation period of about one month, and the entire volume of gas gradually evolved was 552.2 c.c. This mixture consisted of 190.8 c.c. methane and 361.4 c.c. carbon dioxide. The products formed from the 2.0065 g. cellulose consumed included 1.0223 g. fatty acids, .8678 g. carbon dioxide and .1372 g. of methane or a total of 2.0273 g. The slight difference in weight in favor of the

rmentation products falls within the limit of error. These experi-ents show that about one-half of the fermentation products is seous and that the other half consists of acetic and butyric acids.

The Oxidation of Methane, Hydrogen and Carbon Monoxide.—Aside om cellulose, methane may also be produced from various other carbo-drates, organic acids and proteins. Large amounts of methane are us contributed to the atmosphere by swamps, manure heaps and low-ing meadows. In a purely chemical way methane may also be set e from volcanoes and mineral springs. The constant additions of ethane, ethane, hydrogen and carbon monoxide represent a consid-able amount of potential energy. It is important to know, therefore, nether these materials are at all utilized.

That methane may be utilized by bacteria as a source of energy was st shown by Söhngen in 1905. He isolated an organism named by m *B. methanicus* that showed itself capable of growing in inorganic lutions confined over an atmosphere of methane, oxygen and nitrogen. ne methane gradually disappeared and there were formed considerable uantities of organic matter. The ability to oxidize methane has been imed for a number of other organisms by Söhngen and others.

Early observations on the ability of moist soil to cause the oxidation hydrogen are credited to de Saussure (1838). Many years later 892) Immendorff called attention to the same fact. It was not, wever, until 1905 that the oxidation of hydrogen was shown to be a ecific biological process. In that year papers by Söhngen and Kaserer ported experiments wherein inorganic solutions confined under an mosphere of hydrogen, oxygen and carbon dioxide and inoculated with ry small quantities of soil developed a bacterial membrane at the rface. The hydrogen was oxidized and organic matter produced at e expense of the energy set free. The observations just noted have en confirmed by other investigators, by means of mixtures and single ecies of soil bacteria. Finally it should be added here that *B. igocarbophilus* previously isolated by Beijerinck and Van Delden is le, according to Kaserer, to oxidize also carbon monoxide.

The Cleavage and Fermentation of Sugars, Starches and ums.—Sugars (page 163) are a very acceptable source of food and ergy for soil bacteria. A culture solution containing suitable mineral lts and sugar ferments readily when inoculated with a small amount fresh soil. When no combined nitrogen is added, *Azotobacter*, or *B.*

(*Clostridium*) *pasteurianus* (or both), may come to the fore. The cleavage products then include alcohols, organic acids and carbon dioxide. With *B.* (*Clostridium*) *pasteurianus* butyric acid is one of the prominent cleavage products. When combined nitrogen is also added to the culture solution other organisms will develop prominently, notably members of the subtilis group, butyric bacteria, aerogenes, etc. In the soil itself the addition of sugar leads to a very marked increase in number and, if acid production is favored, molds may subsequently become prominent. In general it may be said that butyric, propionic, acetic, formic and lactic acid, and ethyl, propyl, butyl and iso-butyl alcohol are common cleavage products.

In the case of starch, pectins and pentosans, similar conditions hold good. Diastatic enzymes seem to be produced by various bacteria as well, as molds and streptothrices. Members of the subtilis group and *B. fluorescens* seem to be able to transform starch into sugar without difficulty. It needs hardly be added here that the vast quantities of organic acids and of carbon dioxide thus formed must play an important rôle in the breaking down of the mineral constituents in the soil.

Fats and Waxes

Origin and Decomposition.—Plant substances contain varying proportions of fats and waxy materials. In the dry matter of grasses and cereal straw crude fat is usually present to the extent of 1.5 to 2.0 per cent. In hay made from clover and other legumes the proportion of crude fat is rather more than 2 per cent. In cereal grains it may range up to 4 or 5 per cent while in soy beans the content of crude fat is 19 per cent, in germ oil meal 22 per cent and flax seed meal 34 per cent.

Under the influence of enzymes produced by molds, yeasts and bacteria the fatty acids occurring as glycerides are decomposed into glycerin and fatty acids. The extent of fat decomposition, brought about largely by molds in the opinion of some, is shown by numerous experiments with peanut cake, olive press cake, cottonseed meal, almond oil, corn meal, etc. In a number of these experiments *Aspergillus niger* seemed to be particularly efficient in decomposing fat. Analogous decomposition processes may occur in the soil as proved by the experiments of Rubner.

Organic Acids

Source.—The cleavage products of proteins include large quantities amino-acids. The latter are still further transformed and yield a riety of fatty acids. The carbohydrates being present in larger antities than the proteins are still more important as a source of ganic acids. Finally, the fats, gums, and higher alcohols contribute ditional quantities of the latter. Among the more simple acids, etic, propionic, butyric, succinic and lactic are common. The extent acid production was already indicated in connection with cellulose composition by the methane and hydrogen bacilli. Apart from these ganisms, organic acids are formed by nearly every important species soil bacteria; moreover, the tissues of dead plants and animals are t the sole source of organic acids in the soil. According to Stoklasa ditions may occasionally occur in the latter, especially when nospheric oxygen is excluded, that favor the excretion by plant roots appreciable quantities of acetic acid.

Transformation and Accumulation.—Salts of organic acids are table as food for a wide range of soil bacteria. *Azotobacter* will dily make use of acetates, propionates and butyrates. A number of nitrifying bacteria will grow vigorously with citrates as the only urce of organic nutrients. The fermentation of lactates by butyric cteria has been known for a long time. The decomposition of malates, succinates, tartrates and valerates may be accomplished by rious species, and even simple compounds like formates may yield od and energy to certain soil bacteria, among them *B. methylicus* udied by Loew and his associates. It is evident, therefore, that ganic acids are not liable to accumulate in well-ventilated soils. olds, as well as bacteria, destroy them rapidly, and carbonates, rbon dioxide and water are the final products of the decomposition non-nitrogenous organic matter.

Notwithstanding the ready decomposition of the more simple ganic acids in the soil, it is well known that arable soils are frequently id. This acidity is largely due to the so-called "humic acids," ganic compounds whose composition is not well understood. They e composed, to some extent, of rather complex organic acids or of their id salts. Continued cultivation seems to favor the accumulation of ese acid compounds, partly on account of the diminished supply of lime and of other basic materials in older soils. When these soils are

limed the humic acids and acid humates are changed into neutral com pounds and are then subject to more rapid decomposition by micr örganisms. According to the investigations of Blair the average ac soil in Florida requires 1,500 pounds of lime (CaO) per acre to neutrali the acidity to a depth of 84 mm. (9 inches). This means an acidit equivalent to more than one ton of hydrochloric acid per acre. peat and muck soils the acidity is equivalent to many times th amount of hydrochloric acid.

Protein Bodies

Amount and Quality.—The protein content of farm crops th leave residues in the soil is variable, but in all cases quite considerabl Dried corn stalks contain 5 per cent of protein, timothy hay 6 per cer red clover hay 12 per cent or more, alfalfa hay 15 or 16 per cent. Ev wheat and rye straw may contain as much as 3 per cent of protei Cotton-seed meal and other oil cakes, tankage, ground fish, hair a wool waste and dried blood (all used more or less extensively as sourc of nitrogen to crops) are made up in a large measure of prote compounds.

Being derived from plant residues, from microörganic, insect a animal remains, and from fertilizers and manures applied, the nitrog in the soil humus exists, for the most part, in the form of protein co pounds. Hilgard reports the following humus and nitrogen conte as based on the analyses of a large number of samples of humi semi-arid and arid soils.

	(Humus), per cent	(Nitrogen in humus), per cent	(Nitrogen soil), per cent
Arid uplands	0.91	15.23	0.135
Sub-irrigated arid soils	1.06	8.38	0.099
Humid soils from humid and arid regions (California)	2.45	5.29	0.135
Humid soils from other states	7.01	3.78	0.295

Taking the weight of an acre-foot of dry soil at 2,000,000 l (4,000,000 pounds) and multiplying the nitrogen by 6.25 (the fac usually employed for converting nitrogen into protein) we find t protein content of these soils to range from about 11,339 kg. (25,0

pounds) per acre to nearly three times as much. Similarly, the Illinois Experiment Station reports quantities of nitrogen equivalent to 3,175 to 4,989 kg. (7,000 to 11,000 pounds) per acre to a depth of 11.6 cm. (40 inches) in gray silt loams, of the lower Illinoisan glaciation. In the brown silt loams the amount of nitrogen to the same depth is usually more than 4,535 kg. (10,000 pounds) per acre; occasionally it is more than 9,071 kg. (20,000 pounds) per acre. In one instance a black clay loam of the late Wisconsin glaciation is reported to have about 13,154 kg. (29,000 pounds) of nitrogen per acre, to a depth of 11.6 cm. (40 inches). This would be equivalent to more than 81,646 kg. (180,000 pounds) of protein; of course, not all of the nitrogen in the soil exists in the form of protein, some of it occurring as amino-compounds, and a small portion as ammonia and nitrates. Nevertheless, by far the greatest part of it occurs as protein compounds.

The protein compounds of the soil humus must be considered from the standpoint of quality as well as from the standpoint of quantity. It is well known that fresh plant residues are attacked more readily by microörganisms than older plant substances. For this reason soils frequently supplied with fresh organic material supply greater amounts of available food to crops than similar soils whose organic matter consists, largely of older residues.

Carbon-nitrogen Ratio.—The decomposition of organic matter is readily influenced by the relative content of nitrogenous and non-nitrogenous compounds. Substances of animal origin yield relatively and absolutely more available nitrogen in a given length of time than substances of plant origin. The difference noted is due largely to the greater proportion of protein in the animal materials; in other words, to the narrower carbon-nitrogen ratio. On this basis Hilgard attempts to explain the adequacy of the small proportion of humus in arid and semi-arid soils. Because of the narrower carbon-nitrogen ratio the humus compounds in these soils are decomposed with greater rapidity and yield a sufficient amount of ammonia and nitrate to supply the needs of the crop.

But when plant substances alone are considered the statement just made requires qualification. It is true that cotton-seed meal or linseed meal, having a narrower carbon-nitrogen ratio, will decay more readily than corn-meal or wheat flour. It is also true that any given plant substance, as it undergoes decay, will lose in proportion more carbon than

nitrogen. Older humus has, therefore, a narrower carbon-nitroge ratio than humus of recent origin. The former is more resistant t decay, however, than new humus. In a concrete way, on the oth hand, it may be stated that fresh vegetable material of a narrow ca bon-nitrogen ratio will decay more rapidly than fresh vegetable materi of a wide carbon-nitrogen ratio. The reverse, nevertheless is true vegetable materials in advanced stages of decay. Under any giv climatic conditions and in any given soil type, the carbon-nitrog ratio may give important indications only as to the availability of t humus nitrogen. Lawes and Gilbert, as quoted by Hall, found t following carbon-nitrogen ratio in the organic matter of different soil

Cereal roots and stubble	43.0
Leguminous stubble	23.0
Dung	18.0
Very old grass land	13.7
Manitoba prairie soils	13.0
Pasture recently laid down	11.7
Arable soil	10.1
Clay subsoil	6.0

Hall concludes, therefore, that humus with a wide carbon-nitrog ratio is more valuable than humus with a narrow carbon-nitrogen rat since the latter will be attacked more easily by the soil bacteria. Bro and Allison indicate that there might be a possibility of applying n terials of a wide carbon-nitrogen ratio to supply the deficiencies organic matter on the basis that the former may have the same better effect on bacterial activities such as azofication, or non-symbio nitrogen fixation.

The Transformation of Nitrogen Compounds

Ammonification. *Experimental Study.*—By ammonification s meant the production of ammonia by bacteria out of protein substan s or their cleavage products. That ammonia production in the soi s a biological process was first demonstrated by Müntz and Coudon 1893. These investigators showed that no ammonia is formed in ste e soils. They also showed that ammonia may be produced out of ni - genous organic matter by molds as well as by bacteria. Marchal t only confirmed these observations, but proved that various mi - örganisms differ markedly in their ability to produce ammonia. f the several species of bacteria tested by him, *B. mycoides* (one of e

cmmon soil bacteria) proved itself particularly efficient in the breaking
own of nitrogenous materials and the production of ammonia.

Since the publication of these experiments a large number of investi-
gtors, both in Europe and America, have studied ammonia production
iculture solutions as well as in the soil itself. It has been shown that
nder favorable conditions the breaking down of protein compounds and
t formation of ammonia may be very rapid; for instance, in some ex-
periments carried out by Lipman and his associates the following pro-
rtions of nitrogen were transformed into ammonia in the course of
s days:

Dried blood	16.74 per cent
Concentrated tankage	56.66 per cent
Ground fish	47.16 per cent
Cotton-seed meal	4.95 per cent
Bone meal	16.65 per cent
Cow manure, solid and liquid excreta	32.60 per cent
Cow manure, solid excreta	5.39 per cent

The experiments were carried out in equal quantities of soil and with
eiivalent quantities of nitrogen in the different substances. It will
b observed that more than 56 per cent of the nitrogen in the con-
ctrated tankage was transformed into ammonia, whereas under the
sne conditions cotton-seed meal yielded less than 5 per cent.

Mechanism of Ammonia Production.—The relatively large protein
nlecules are readily broken into larger or smaller fragments. This
ny be accomplished by purely chemical means, as, for instance, by
bling with acids or alkalies, or by biological activities. Among the
fit cleavage products albumoses and peptones are quite prominent.
Tese in turn undergo further cleavage and the various amino-acids
al their derivatives, as well as ammonia, make their appearance. In
s far as the different species of bacteria are concerned, ammonia pro-
d:tion seems to depend, to a marked extent, on the ability to secrete
p teolytic enzymes. With the aid of such enzymes the proteins are
nre readily hydrolyzed and further changed into amino- and hydroxy
ads, ammonia and carbon dioxide.

Influence of Soil and Climatic Conditions.—Ammonia production in
t soil is affected by (*a*) its mechanical and chemical composition; by
(the amount and distribution of rainfall; by (*c*) the prevailing tem-
patures; by (*d*) fertilizer treatment; and by (*e*) methods of tillage and

cropping. The mechanical composition of the soil influences the pr portion of aerobic and anaerobic species, while the chemical compos tion, particularly that of the humus, influences the rate of multiplic tion and the character of the chemical transformation accomplishe It is well known, for example, that additions of fresh organic matt intensify the rate of decomposition of the soil humus, and, likewis ammonia production as has been already demonstrated by Breal. In more general way it was proved by Lipman and his associates tha with a constant bacterial factor, ammonia production in soils varies wi the chemical and mechanical composition of the latter. In some these experiments 100 g. portions of different soils were each mixed wi 5 g. of dried blood, sterilized in the autoclave, cooled and inoculat with equal quantities of infusion from fresh soil. The followi amounts of ammonia nitrogen were produced in six days:

Soil	Ammonia nitrogen found
A	31.62 mg.
B	68.29 mg.
C	117.06 mg.
D	107.16 mg.
E	156.47 mg.

With all other factors constant, chemical and mechanical differenc in the soil used were responsible for striking variations in ammor production, as indicated by the figures given above.

The influence of temperature and moisture conditions is fully important as that of the chemical and mechanical composition of t soil. The following data secured by Lipman may be cited in tl connection as showing the effect of moisture:

One-hundred-gram quantities of air-dried soil were each mixed wi 3 g. of dried blood and varying amounts of water added. The ammoi formed was distilled off and determined at the end of eight da The amounts of ammonia nitrogen found were as follows:

Water added	Ammonia nitrogen found
0 c.c.	4.13 mg.
1 c.c.	4.13 mg.
3 c.c.	5.40 mg.
5 c.c.	10.64 mg.
7 c.c.	26.37 mg.
10 c.c.	49.57 mg.
12 c.c.	70.71 mg.
15 c.c.	93.90 mg.

It appears, therefore, that ammonia production in soils rises or falls a the rainfall or irrigation is increased or decreased, or as the soil water i more or less thoroughly conserved by proper methods of tillage. In te same way, seasons of high temperature favor ammonification while sasons of low temperatures discourage it. This point is well illustrated b the observations of Marchal that at 0° to 5° only traces of ammonia vre formed in his culture solutions; that at 20° ammonia production vs quite marked, and that at 30° the maximum was reached. Moreer, apart from the seasonal variations in any one locality, there is a vle range in ammonia production, as we pass from the torrid to the tnperate and from the latter to the frigid zones.

Species and Numbers.—Ammonia production is a function common t most soil bacteria. Already in the earlier experiments of Marchal, seenteen out of the thirty-one species tested were found capable of pducing ammonia. Prominent among these ammonifiers were *B. mcoides*, *B.* (*Proteus*) *vulgaris*, *B. mesentericus vulgatus*, *B. janthinus*, a *B. subtilis*. Of a considerable number of soil bacteria tested by Cester all but one were observed to produce ammonia. In Gage's eeriments with sewage bacteria, seventeen out of twenty species tted proved to be ammonifiers. Similarly, a number of species tested b the writer, among them *B. coli*, *B. cholerœ suis*, *B.* (*Proteus*) *vulgaris*, *Bsubtilis*, *B. megaterium*, etc., all produced ammonia in meat infusions. Amass of additional data, accumulated by different investigators, fnish further proof that ammonia production is a common function o soil bacteria.

The more prominent ammonifiers, including members of the *B. stilis* group and certain streptothrices, are numerically important in a arable soils. Their numbers are affected, however, by the amount a composition of the soil humus. It has been found, for instance, th additions of straw and of strawy manure increase markedly the nmbers of *B. subtilis* and of other members of the group. An increase in he numbers of certain ammonifiers is caused also by additions of lie or of green manure. For example, in experiments carried out by Lman and his associates portions of fertile soil inoculated with *B. mcoides* were found to contain, a month later, 2,000,000 of bacteria per g. f soil. In similar soil portions that had also received additions of grass the number was twice as great.

Relative Efficiency of Different Species.—In Marchal's experimen already referred to, the species employed showed marked differences their ability to produce ammonia out of egg albumin. The followir proportions of the protein nitrogen were converted into ammonia twenty days:

B. mycoides.....	46 per cent	*B. subtilis*.....	23 per ce
B. (Proteus) vulgaris.....	36 per cent	*B. janthinus*.....	23 per ce
B. mesentericus vulgatus..	29 per cent	*B. fluorescens putidus*.....	22 per ce
Sarcina lutea.....	27 per cent	*B. fluorescens liquefaciens* .	16 per ce

Furthermore, apart from the variations from species to species, diff ences have been observed by Marchal and many other investigatc between one strain and another of any single species isolated from t same or different soils. It must be remembered, therefore, that in t study of ammonification in soils and culture solutions, due consider tion should be given to differences in *physiological efficiency* as they a manifested by strains and species of microörganisms.

Apart from the ammonifying bacteria already mentioned there i group of organisms studied by Müller, Pasteur, van Tieghem, Leul Miquel, Beyerinck and others. These are the so-called urea bacter capable of intensive transformation of urea and allied compounds ir ammonium carbonate, by means of the enzyme urease.

$$\begin{array}{l} NH_2 \\ | \\ CO + 2H_2O = (NH_4)_2CO_3 \\ | \\ NH_2 \end{array}$$

Morphologically these organisms include spherical and rod for spore-bearing and non-spore bearing species. Most of the urea bacte are particularly prominent in the transformation of animal manures

Ammonifying Efficiency.—Lipman and Burgess have found marl differences in the ammonifying efficiency of fifteen organisms in p cultures using peptone, bat guano, sheep and goat manure, d blood, tankage, cottonseed meal and fish guano. The nature of soil as well as the nature of the nitrogenous material markedly mo an organism's ammonifying power. *B. tumescens* on the whole appear have been the most efficient organism tested. Comparing these findi

th those of Marchal the former have obtained results in soils, while e latter's work was with solution cultures, the application of which soil conditions is not always permissible. In point of fact the amnifying efficiency of organisms is greater in sandy soil and possily in others than in solutions, as Lipman and Burgess have obtained transformation of 41.98 per cent of peptone in nitrogen and 36.06 r cent of bat guano nitrogen into ammonia by *Sarcina lutea* and *mycoides*, respectively, in twelve days at temperatures between ° and 30°., while Marchal obtained similar transformations in irty days at 30°. in albumen solutions.

It is also of interest to note that investigations with soil fungi have vealed the fact that certain species are even more efficient amnifiers than *B. mycoides*. McLean and Wilson, Waksman, Coleun and Kopeloff have worked with organisms like *Trichoderma eningi* which is capable of transforming more than 50 per cent of e nitrogenous material added in such experimentation.

NITRIFICATION. *Experimental Study.*—The term nitrification refers the oxidation either of ammonia or of nitrites to nitrates. In a oader sense nitrification may be defined as the production of nitrates m decomposing organic matter. Saltpeter or niter, the terms rmerly applied to potassium nitrate, possessed, for a long time, a culiar interest because of its relation to gunpowder. Whether it be ue or not that gunpowder was known to the Chinese before the beaning of the present era, there is no doubt that for several centuries played an important part in the political and economic history of rope. The large quantities of gunpowder consumed in the almost essant wars created a steady demand for saltpeter that was not adily met by the saltpeter refiners of India, Hungary and Poland. uropean nations, particularly France, were therefore thrown on their n resources and were forced to develop the domestic production of ltpeter. The industry came under government control and experts re appointed to study the so-called saltpeter plantations and the nditions affecting the appearance and increase of nitrates in comst heaps and in the soil. Much knowledge was thus gained about rification even though it was not suspected that living organisms re concerned in the process.

With the rapid development of chemistry in the latter half of the hteenth century a nearer approach was made to the understanding

of the true character of nitrification. The observations of Cavendis in 1784 that potassium nitrate is formed when electric sparks are passe through air confined over a solution of potassium hydrate formed th starting point for various theories that attempted to account for nitrat formation on the basis of purely chemical reactions. The formation nitric acid and of its salts was thus assumed to be due to electric di charges in the atmosphere, to combustion processes in nature, or to th oxidation of organic matter and of calcium, magnesium, iron and ma ganese compounds in the soil. Much credence was given to the latt explanation because of the almost universal occurrence of nitrates arable soils.

The first indication that nitrate production in the soil and in d caying organic matter is due to biological activities was given Pasteur in 1862. A few years later Müller expressed his belief in th biological origin of nitrates and nitrites in sewage and drinking wate It was not, however, until 1877 that the true character of nitrificati was made clear. In that year Schloesing and Müntz demonstrat that dilute solutions of ammonia could be changed into nitrate by bei passed slowly through long tubes filled with soil. The amounts nitrate nitrogen found in the leachings corresponded almost exact to the amount of ammonia nitrogen used up. When the soil in th tubes was first sterilized by heating or by means of chloroform and oth germicides, the ammonia passed through unchanged. But when so sterilized by heat or chloroform were reinfected with small quantiti of fresh soils nitrification again proceeded in a normal manner.

The biological nature of nitrification having been thus establish numerous investigators tried to isolate the specific organisms in pu culture. A large amount of work in this direction was done Schloesing and Müntz, Celli and Marino-Zuco, Munro, Warington, t Franklands and many others. A large number of bacteria, yeasts a molds were tested with negative results. Warington, who gather a great mass of valuable information about nitrification, almo succeeded in securing pure cultures of nitrifying bacteria. Finall Winogradski showed in 1890 not only that nitrification is caused specific bacteria, but explained also why the others failed in securi pure cultures. He proved that these organisms do not develop coloni on the ordinary gelatin and other organic media, a fact whose recc nition was largely responsible for his successful solution of the proble

he medium subsequently employed by him consisted of silica jelly roperly supplied with inorganic nutrient salts. After him other in-stigators proved that agar, deprived of its soluble organic matter, psum and sandstone disks, filter-paper pads, etc., could be used fectively as solid media.

Nitrous and Nitric Bacteria.—Winogradski's investigations led to e conclusion, foreshadowed by the earlier work of the Franklands and arington, that the oxidation of ammonia proceeds in two stages, viz.,

$$(1)\ 2NH_3 + 3O_2 = 2HNO_2 + 2H_2O$$
$$(2)\ 2HNO_2 + O_2 = 2HNO_3$$

The organisms oxidizing ammonia to nitrites, and designated as rous or nitrite bacteria, were called by Winogradski *Nitrosomonas* d *Nitrosococcus*. The former include species or varieties isolated m soils in Europe, Asia and Africa, and the latter those isolated from ils in America and Australia. The organisms oxidizing nitrites to rates and known as nitric or nitrate bacteria, were included by inogradski in the genus *Nitrobacter*.

Apart from these bacteria there is an organism, according to Kaserer, at can oxidize ammonia directly to nitrate. He named it *B. nitrator*. e reaction is illustrated by the following equation:

$$NH_3 + H_2CO_3 + O_2 = HNO_3 + H_2O + CH_2O - 41 \text{ Cal.}$$
$$CH_2O + O_2 = H_2CO_3 + 132 \text{ Cal.}$$

Enough energy for the completion of the reaction is obtained by the idation of the formaldehyde (CH_2O). Beyond the preliminary nouncement of Kaserer's there are no experimental data to prove e existence of this organism, even though other evidence of an direct nature may be construed to lend support to his theory. t whether it be proved or not that ammonia may be oxidized nitrate by a single species, it is evident that the number of species ncerned in nitrate production is relatively small.

Relation to Environment.—The conditions that affect nitrate forma-n in soils may be classified under the following heads: (*a*) supply of ygen; (*b*) range of prevailing temperatures; (*c*) amount and dis-bution of moisture; (*d*) quantity of lime and of other basic materials; quantity of soluble mineral salts; (*f*) character and amount of

organic matter; (*g*) presence of toxic substances; (*h*) physiologic efficiency of the nitrifying bacteria.

The rapid disappearance of organic matter from sandy soils is due i large measure to their better aeration. On the other hand, the decon position of vegetable and animal substances in heavy, ill-ventilated soi is materially retarded by the limited supply and very gradual renewal oxygen. An intimate relation exists here between air and water in th the latter replaces the former to a more marked extent in heavy than i light soils. The influence of both aeration and the range of moisture illustrated by an experiment of Lipman's in which equal quantities soil were kept in large boxes under different moisture conditions. the end of a year the following quantities of nitrate nitrogen we found:

Moisture content	6.52 per cent	14.75 per cent	18.62 per cent	22.05 per cent	22.12 per ce
Nitrate nitrogen found	697 mg.	823 mg.	720 mg.	Trace	Trace

In examining the figures recorded above, we find that moisture was t controlling factor in the development of the nitrifying bacteria, wh the proportion of water in the soil was 6.52 per cent. As the amount water increased to 14.75 per cent there was a marked increase in t amount of nitrate produced. Beyond that, however, the further i crease in the amount of water began to limit the supply of oxygen, a the production of nitrate nitrogen with 18.62 per cent of water in t soil was somewhat decreased. A still further addition of water up 22.05 per cent led, practically, to saturation, and the encouragement reduction rather than oxidation processes. Hence, no nitrate was lowed to accumulate in the soil. The data in question thus help explain why care was taken, on salt-peter plantations, to keep t compost heaps moist, yet not too wet.

The influence of temperature on nitrate formation has been observ by many investigators. Already Schloesing and Müntz recorded th at 5° nitrification is quite feeble, at 12° marked and at 37° at its be Other investigators have obtained substantially the same results, exce that the optimum has been found to be considerably lower, often b tween 25° and 30°. Under field conditions nitrification seems to ta place at relatively low temperatures, as is indicated by the ra

idation of ammonium salts in the Rothamsted experiments in Eng-
nd; and the rapid decay and nitrification of clover and of other
gume residues in the experiments at the New Jersey Experiment
ation. These facts have, therefore, an important bearing on the
trogen feeding of crops in tropical, subtropical and temperate zones.

The influence of lime and of other basic substances including the
rbonates of magnesium, potassium and sodium, and of the oxides of
on is of far-reaching importance in all nitrification processes. It is
ell known that applications of magnesian and non-magnesian lime,
arl or wood ashes promote nitrification in the soil and in compost
aps, a fact that was well recognized by the ancient niter refiners. The
vorable action of lime is readily explained by its ability to neutralize
ganic and mineral acids and to render, thereby, the soil reaction
vorable for the rapid growth of ammonifying, as well as of nitrifying
cteria. Furthermore, the reserve of basic material serves to neutral-
e the acid formed by the bacteria and prevents thus the accumulation
an undue amount of acidity.

The rôle of certain mineral salts in promoting nitrification is quite
gnificant. Small amounts of sodium chloride have been found to favor
trification in the experiments of Pichard and also those of Lipman.
he former showed also that sulphates not only promote nitrification,
ut that different sulphates display marked variations in this respect.
n the same manner nitrate formation was shown to be favorably
fected by phosphates in bone meal, Thomas slag, and acid phos-
hates. Generally speaking, therefore, nitrifying bacteria are stimu-
ted in their development by a proper supply of available mineral
utrients.

The exact relation of organic matter in the soil to the activities of
trifying bacteria is but beginning to be properly understood. Earlier
bservations made it manifest that heavy applications of animal
anures, or of green manure may not only retard nitrification, but may
ctually cause the disappearance of a part or of all of the nitrate in the
oil. Subsequent experiments by Winogradski and by Winogradski
nd Omelianski showed that in pure cultures the presence of even slight
mounts of soluble organic matter may depress or even suppress the
evelopment of the nitrifying bacteria. It was, therefore, concluded
y these authors that relatively small amounts of soluble organic
natter may inhibit nitrification. These conclusions, based on the

study of liquid cultures only, were given a very broad application b many writers on agricultural topics. More recent experiments mak it certain, however, that in the soil itself small amounts of solubl organic matter, *e.g.*, dextrose, are not only harmless, but may reall stimulate nitrification. It was shown, likewise, that humus an extracts of humus may, under suitable conditions, stimulate nitrifica tion to a very striking extent.

Certain substances in the soil may exert a toxic effect on nitrifyin bacteria. Ferrous sulphate, sulphites and sulphides may thus act ir juriously, as may also calcium chloride and excessive concentrations c sodium carbonate, sodium bicarbonate, sodium chloride, magnesiur sulphate, etc. Injury by ferrous compounds, as well as by organi acids, is not uncommon in low-lying fields and bogs; while injury fro excessive concentration of soluble salts may occur in the so-calle alkali lands.

Finally nitrification in the soil should be considered from the stanc point of the organisms themselves. There is no doubt that continue growth under extremely favorable conditions leads to the develo ment in the soil of nitrifying bacteria, possessing a very marked phy siological efficiency. On the other hand, in ill-aerated, sour soils th environment would depress the physiological efficiency of the nitrif ing bacteria. Differences are thus undoubtedly established unde actual field conditions, as is made probable by the variable behavic of soils from different sources when used as inoculating material i recently reclaimed or peat swamp lands.

Accumulation and Disappearance of Nitrates.—As shown above, th rate of formation of nitrates in the soil is dependent upon moistur temperature and aeration, as well as on the presence of organic matte and basic substances. On the other hand, the accumulation of nitrate depends, under any given conditions, largely on the character of th growing crop. Observations on the rain gauges at Rothamsted showe an average annual loss 14 kg. (31.4 pounds) of nitric nitrogen per ac in the drainage water from uncropped soil. In one of King's exper ments, land that had been fallowed contained 137 kg. (303.24 pound of nitric nitrogen per acre, to a depth of 4 feet. Adjoining croppe land contained only 26 kg. (57.56 pounds) of nitric nitrogen per ac to the same depth. Stewart and Greaves found in limestone soil i Utah 64 kg. (142 pounds) of nitric nitrogen per acre, under corr

pounds under potatoes, and only 12 kg. (27 pounds) under alfalfa. nder the same conditions fallow land contained 74 kg. (165 pounds) nitric nitrogen per acre. The smaller amount of nitric nitrogen found der alfalfa bears out the observations already made by a number of her investigators that the accumulation of nitrates under legumes is naller than it is under non-legumes. While several explanations have len offered to account for this fact, it is generally agreed that legumes similate nitrate nitrogen more rapidly than non-legumes. Unusual cumstances may favor, at times, the accumulation of quantities of rate large enough to destroy all vegetation. It is reported, for stance, by Headden that he has found in limited areas in Colorado as uch as 90,718.5 kg. (100 tons) of nitrate per acre foot of soil.

The amount of nitrate nitrogen in the soil is influenced by the grow- g crop not alone because of the nitrogen absorbed by the latter, but cause of the moisture relations as affected by growing plants. It is ite apparent that a large crop dries out the soil more rapidly than a all crop. When the soil moisture is sufficiently depleted, nitrifica- n stops and the further accumulation of nitrates becomes impossible, ile their disappearance is hastened by the constant demands of the p. The disappearance of soil nitrates is, likewise, hastened by the ching action of rain and by certain species of bacteria that transform em into other nitrogen compounds.

DENITRIFICATION. *Experimental Study.*—Denitrification may be fined as the reduction of nitrates by bacteria, involving the evolu- n of nitrogen gas or of nitrogen oxides. In a more general way, nitrification has been defined as the partial or complete reduction of rates by bacteria. The term direct denitrification has been sug- sted for complete reduction, and indirect for the partial reduction nitrites or ammonia. The term denitrification should not be em- yed to designate losses of nitrogen gas due to the oxidation of mmonia, or to the disappearance of nitrates following their conversion o proteins by microörganisms.

The reduction of nitrates in the presence of fermenting organic tter was noted by Kuhlmann as early as 1846. The same fact was corded many years later by Froehde and by Angus Smith. In 1868 hoenbein expressed the belief that nitrates may be reduced to nitrites fungi. For more than a decade after that, data were rapidly accu- ulating in support of Schoenbein's contention, until in 1882 Gayon

and Dupetit made it certain that nitrate reduction with the evolution of nitrogen gas may be caused by a "ferment." Finally, in 1886, the same investigators described two organisms, *B. denitrificans* α, and *B. denitrificans* β, capable of completely reducing nitrates. Subsequently the studies of Giltay and Aberson, Burri and Stutzer, Severin, van Iterson, Jensen, Beyerinck and of many others not only greatly increased the number of known denitrifying bacteria, but added much to our knowledge concerning the development and activities of these organisms. It has been shown that a very large number of species can reduce nitrates to nitrites and ammonia; moreover, a considerable number of organisms are already known that can cause the complete destruction of nitrates with the evolution of nitrogen gas or nitrogen oxides. The following reactions illustrate diagrammatically the complete or partial reduction of nitrates.

$$2HNO_3 = 2HNO_2 + O_2$$
$$HNO_3 + H_2O = NH_3 + 2O_2$$
$$4HNO_2 = 2H_2O + 2N_2 + 3O_2$$

In the soil, manure or other culture media the denitrifying bacteria which are, for the most part, aerobic develop also under anaerobic conditions and transfer the oxygen of nitrates and nitrites to carbon compounds. This is illustrated by the equations suggested by van Iterson:

$$5C + 4KNO_3 + 2H_2O = 4KH\,CO_3 + 2N_2 + CO_2$$
$$3C + 4KNO_2 + H_2O = 2KH\,CO_3 + K_2CO_3 + 2N_2$$

When nitrates are reduced to nitrites in the presence of amino compounds, or even of ammonium compounds, elementary nitrogen may escape as shown by the following reactions:

$$C_2H_5NH_2 + HNO_2 = C_2H_5OH + N_2 + H_2O$$
$$NH_4Cl + KNO_2 = KCl + 2H_2O + N_2$$

An organism has been described by van Iterson that can decompose nitrates in the presence of cellulose:

$$5C_6H_{10}O_5 + 24KNO_3 = 24KHCO_3 + 6CO_2 + 12N_2 + 13H_2O$$

Still more interesting is *Thiobacillus denitrificans* described by Beyerinck as capable of reducing nitrates in inorganic media. The nitrate oxygen is used to oxidize elementary sulphur:

$$6KNO_3 + 5S + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 3N_2$$

The *Actinomyces* reduce nitrates to nitrites, but they do not cause ay loss of free nitrogen, for the nitrites are utilized by the organisms, ad complete denitrification does not take place. Thus these organ- ins may prevent the leaching out of nitrates and nitrites in the soil, c the active denitrification by other organisms.

Relation to Environment.—Nitrate reduction is favored by insuffi- cnt aeration, as well as by an abundance of readily decomposable cganic matter. In fine-grained, compact soils nitrate formation and rrate reduction may alternate, depending upon the more or less cmplete replacement of soil air by water. Similarly, in soils receiving ecessive amounts of animal manure denitrifying bacteria may cause te reduction of nitrates. In greenhouse soils excessive moisture, as vll as excessive amounts of organic matter, may be present and may pevent the accumulation of nitrates. It has also been shown by Iklevski that, contrary to opinions previously held, denitrification ny occur in manure heaps. In the better aerated surface portion of nure heaps conditions are favorable for the oxidation of ammonia t nitrites and nitrates. The nitrous acid may combine with ammonia t form ammonium nitrite, the latter decomposing, spontaneously, into ter and nitrogen gas. It is very likely, also, that the nitrites and rrates are reduced by the denitrifying bacteria in manure. On the cer hand, in manure kept moist under the feet of cattle nitrite and rrate formation is prevented and losses by denitrification are not lely to occur.

The economic significance of denitrification was overestimated at e time, on account, largely, of the assertion of Wagner in 1895 that i all soils receiving applications of horse manure, the nitrates in the sl itself as well as those added in commerical fertilizers are almost ctain to be destroyed by denitrification. Subsequent experiments l many investigators demonstrated that under field conditions, deni- tfication is a factor of slight moment; however, in the greenhouse, i the manure heap (under certain conditions) and in market gardening ere manure is used at the rate of 45,359 kg. to 54,431 kg. (50 to 60 ns) per acre, the danger of denitrification is real.

Analytical and Synthetical Reactions

Amount of Bacterial Substance in the Soil.—Various decom- sition processes in the organic matter of the soil may be designated

as analytical in that protein, carbohydrates and fats are split into mor simple compounds. At the same time, the microörganisms concerne in the decomposition processes multiply very rapidly and fashion th complex compounds of their cell-substance out of the simple cleavag products in their medium. In other words, analytical and synthetic reactions proceed hand in hand in the soil.

While it is not definitely known how large a proportion of the so humus consists of the dead and living cells of microörganisms ther is a mass of indirect evidence to show that these cells form a very cor siderable proportion of the total quantity of organic substances in th soil. For instance, it has been demonstrated that a large proportion c the dry matter of solid animal fæces may consist of bacterial cells. A various times and by different investigators the proportion of bacteri substance has been estimated at from 5 to 20 per cent or more of th total dry weight of fæces. A heavy application of barnyard manur may introduce, therefore, several hundred pounds of bacterial cells p acre of soil. Moreover, because of the extensive changes in the so humus itself, as is evidenced by the rapid formation of nitrates, larg masses of bacterial substances are constantly being formed and di integrated.

Availability of Bacterial Matter.—Substances of microörgani origin are decomposed more or less rapidly, according to their con position. The extent of transformation under favorable conditions indicated by an experiment performed by Beyerinck and van Delde in which 50 per cent of the nitrogen in *Azobacter* cells was transforme into nitrate in seven weeks. On the other hand, the humus of peat an muck soils, or that of worn-out soils, may contain microörganic residu of so inert a character as to yield but little available nitrogen t crops.

Transformation of Peptone, Ammonia and Nitrate Nitroge —The cleavage of protein compounds into peptones, amino-acids an ammonia, and the oxidation of the latter into nitrites and nitrates, ma be properly included among analytical reactions. It should not b forgotten, however, that in the accompanying synthetical reactions th compounds just mentioned may be transformed back into comple proteins. It happens, thus, that large quantities of the availab nitrogen compounds may be withdrawn from circulation by micr örganisms that use these as building material. Under extreme co

ions microörganisms may become serious competitors of higher nts for available nitrogen food.

Manure stored in heaps not infrequently deteriorates in quality, en when losses by leaching are excluded. This deterioration is largely e to the change of the water-soluble ammonia and amino-compounds o insoluble protein substances. While the extent of the change into otein compounds is variable it may range from less than a tenth of the ter soluble material to more than three-quarters or four-fifths of it. so in the soil the same processes take place, but not so intensively. A ge number of species of molds and bacteria have been isolated and ted as to their ability to transform ammonia, amino- and nitrate rogen into protein compounds. Among the more recent investi-tions in this field those of Lemmermann and his associates testify that three weeks 5 to 6 per cent of the nitrate added to the soil was changed o protein. In the presence of barnyard manure the proportion nsformed was increased to 15 per cent. In the case of ammonium mpounds the transformation may be even more far-reaching, amount-g, at times, to more than 25 to 30 per cent of the material originally sent. Generally speaking, molds will assimilate ammonia nitrogen re readily while bacteria and algæ will assimilate nitrate nitrogen preference. However, the preference of molds for ammonia nitrogen often more apparent than real, because of the rapid formation of d residues in culture media rich in certain ammonium compounds. nilarly, some species of bacteria will assimilate ammonia nitrogen preference to nitrate nitrogen.

CHAPTER III

FIXATION OF ATMOSPHERIC NITROGEN

The Source of Nitrogen in Soils

Early Theories.—When chemistry had made sufficient progre to allow the analysis of soils and plants it was recognized that nitroge is always present in both. It was also recognized that the soil nitroge is almost wholly confined to the surface portion and is evidently atmospheric origin, since the unweathered, underlying rock is devo of this constituent. The vast accumulations of nitrogen, known exist in all arable soils, were ascribed, therefore, to the residues of ma generations of plants; and the assumption seemed to be justified th the atmosphere, 79 per cent of whose bulk consists of nitrogen gas, the direct source of this element to plants. It was not long, howev before plant physiologists demonstrated experimentally that nitrog gas as such could not directly serve as food for plants. There th arose one of the most interesting and, for a long time, one of the mo puzzling problems in agricultural research. Among the earlier i vestigators de Saussure believed, at the beginning of the nineteen century, that nitrogen is taken up from the soil in combined for Liebig in 1840 advanced his well-known "mineral theory" accordi to which plants secured their nitrogen from the air, in the form ammonia. He assumed, thus, that plants cannot use elementa nitrogen, and that the supply of atmospheric nitrogen in the form ammonia was great enough to meet the needs of growing vegetati The latter view was not accepted by Lawes and Gilbert of the Ro amsted Station in England. By a series of elaborate and carefu controlled experiments they demonstrated in 1858 that nitrogen in elementary form cannot be used by plants. They further demonstra that the amount of combined nitrogen brought down in the form ammonia, nitrites and nitrates, by atmospheric precipitation was slight when compared with the quantities annually removed by cro Hence the problem as to the source and maintenance of combi nitrogen in the soil seemed to be more puzzling than ever.

Chemical and Biological Relations.—The second and third quarters of the nineteenth century saw the birth of a number of theories dealing with this problem. It was suggested that nitrogen compounds may be formed in the soil by the oxidation of nitrogen to nitric acid. Compounds of iron, manganese and lime were supposed in some way to make such oxidation changes possible. It was likewise suggested that nascent hydrogen may be generated in the decomposition of organic matter in the soil, and reacting with elementary nitrogen, may give rise to ammonia. The various hypotheses were not supported by experimental proof; moreover, the situation was complicated by the knowledge, based on empirical observations, that crops of the legume family seemed to be more or less independent of the supply of combined nitrogen in the soil. Indeed, clovers and other legumes had, apparently, the ability to increase the content of combined nitrogen in the soil as was indicated by the experiments of Boussingault and of Lawes and Gilbert. Finally, the mystery was solved by the investigations of Berthelot and Hellriegel and Wilfarth who furnished the proof that elementary nitrogen may be utilized by plants when certain biological relations are met. These relations involve the presence and activities of microörganisms that by themselves, or in conjunction with higher plants, make available to growing vegetation the great store of atmospheric nitrogen.

Non-symbiotic Fixation of Nitrogen

Historical.—Non-symbiotic nitrogen fixation, or *Azofication*, has already been defined as the production of nitrogen compounds out of atmospheric nitrogen by bacteria independently of higher plants. The part played by bacteria in this process was not recognized until 1885, when Berthelot published some of his data on the accumulation of combined nitrogen in uncropped soils. His results seemed to explain a number of scattered observations, made since the middle of the century, of the apparent increase of the nitrogen content of cultivated soils.

While Berthelot's experiments proved that the nitrogen gains occurred only in unsterilized soils and were, therefore, due to microörganisms, it remained for Winogradski to demonstrate, in 1893, that the formation of nitrogen compounds by certain types of bacteria may be accomplished in culture media nearly or quite devoid of com-

bined nitrogen. Soon after that he succeeded in isolating his organism in pure culture, and described them as anerobic bacilli allied to those of the butyric group. In 1901 our knowledge of *Azobacteria* was enriched by Beyerinck's discovery of a group of large, obligate aerobic bacteria that he designated as *Azotobacter*. Since that date it has been found that the ability to fix atmospheric nitrogen is possessed also by certain molds and by various species of bacteria. However, this ability is not only extremely variable, but is also very feeble as compared with that of the members of the two groups described by Winogradski and Beyerinck. These two groups may, therefore, be designated as including the nitrogen-fixing bacteria par excellence.

Anaerobic Species.—The species isolated by Winogradski was named by him *B.* (*Clostridium*) *pasteurianus* (Fig. 119). It was found

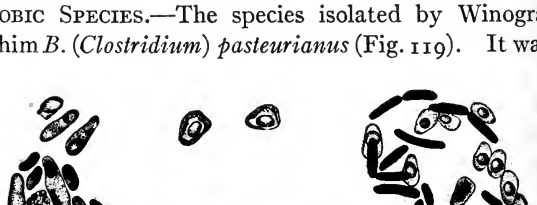


Fig. 119.—*B.* (*Clostridium*) *pasteurianus*, a non-symbiotic nitrogen-fixing organism. (*After Winogradski from Lipman.*)

grow readily under anaerobic conditions in culture solutions containing dextrose and the necessary mineral salts, but no combined nitrogen. The products of growth included protein, butyric and acetic acids, carbon dioxide and hydrogen. In the presence of other bacteria *B.* (*Clostridium*) *pasteurianus* was found to develop also under aerobic conditions. Subsequently studies by Winogradski and other investigators showed that *B.* (*Clostridium*) *pasteurianus*, and varieties of this species are very widely distributed in cultivated soils. More recently Bredeman made a thorough and extended study of anaerobic *Azotobacteria* and demonstrated their almost invariable presence in a large number of soil samples from Europe, Asia and America. In his opinion they correspond more or less closely to *B. amylobacter* described many years before by van Tieghem.

Aerobic Species.—A more or less pronounced power to fix atmo-

peric nitrogen is apparently possessed by a considerable number of arobic species. Lipman has demonstrated the fixation of small amounts of nitrogen by *Ps. pyocyanea* and Löhnis secured similar results wh *Bact. pneumoniæ*, *B. lactis viscosus*, *B. radiobacter* and *B. pdigiosus*. Gottheil has detected fixation by *B. ruminatus* and *B. suplex;* Pillai has described a nitrogen-fixing aerobic bacillus, *B. nilabarensis;* Westermann studied a similar organism that he named *B. ducus;* while Beyerinck and van Delden observed, some years earlier, tt certain strains of *B. mesentericus* could fix relatively large amounts onitrogen. Similarly *Ps. radicicola* has been found to possess a slight, b nevertheless an appreciable power to fix elementary nitrogen in c ture solutions or in the soil.

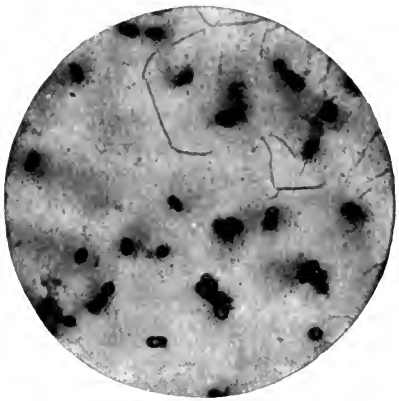


FIG. 120.—*Azotobacter vinelandi*, a non-symbiotic nitrogen-fixing organism. (*After Lipman.*)

But while nitrogen fixation among aerobic soil bacteria is not as ucommon as was at one time supposed, this function is so feeble and sovariable in most instances, as to be of negative, or, at best, of doubtfueconomic significance. On the other hand, the aerobic, *Azotobacter*, fit described by Beyerinck in 1901, may be regarded not only as possing a very pronounced ability to fix atmospheric nitrogen, but as plying a rôle of some moment in maintaining the supply of combined nogen in the soil.

To the two species of *Azotobacter*, *A. chroococcum* and *A. agilis* dcribed by Beyerinck and van Delden, Lipman, added *A. vinelandii*

(Fig. 120), *A. beyerincki* and *A. woodstownii*, and Löhnis and Westerman *A. vitreum*. Of these species *A. chroococcum* and *A. beyerincki* are mo common and are widely distributed in cultivated soils of Europe an America, and probably also of the other continents. They are abse in acid soils deficient in humus, and most common in limestone regio and in irrigated soils rich in mineral salts. Their food requirements a covered by solutions containing potassium phosphate, magnesiu sulphate, calcium chloride and ferric sulphate, and some organ nutrient, such as dextrose, saccharose, xylose, mannit, acetate, pr pionate, butyrate, malate, ethyl alcohol, etc. An alkaline or neutr reaction and the presence of salts of iron are essential for the vigoro development of *Azotobacter*, while humates have been shown l Krzemieniewski to exert a stimulating influence on the growth of the organisms, even though not acting directly as a source of food a energy. As shown by Lipman and others, *Azotobacter* may gain increased power of fixing atmospheric nitrogen in the presence of oth organisms. It is resistant to drying, notwithstanding the fact that produces no spores, and has been successfully isolated from soil sampl that had been kept in a dry state for several years. For some reas it may be detected in the soil most readily in the fall and win months.

As to the nitrogen-fixation by fungi, it has been shown elsewh that the evidence is, if anything, of a negative character. So algæ are able to fix atmospheric nitrogen, especially those that li symbiotically with azotobacter.

Energy Relations.—In the fixation of nitrogen by bacteria t necessary energy for the process is furnished by the carbohydrat organic acids, alcohols or other organic nutrients employed in t culture media. Since any given quantity of organic nutrient posses a definite amount of potential energy the fixation of nitrogen is nec sarily limited by the supply of such potential energy. This limitati was already recognized by Winogradski in his experiments with (*Clostridium*) *pasteurianus*. For every gram of dextrose used up th was produced, on the average, 2 to 3 mg. of combined nitrogen. In experiments of Bredeman with *B. amylobacter*, and of Pringsheim w "*Clostridium americanum*" the amounts fixed were, at times, c siderably larger. On the whole, however, it has been proved b number of investigators that *Azotobacter* can fix much larger quanti

nitrogen than the anaerobic bacilli. The extended investigations Lipman showed that *A. vinelandii* has the ability to fix more nitrogen r unit of organic nutrient consumed than either *A. chroococcum* or *beyerincki*. Under favorable conditions *A. vinelandii* may at times 15 or even 20 mg. of nitrogen per g. of mannit used up. Krzeeniewski found in experiments with *A. chroococcum* that additions humates to the culture solutions increased the nitrogen fixed from a ximum of 2.4 mg. to a maximum of 14.9 mg.

The practical bearing of the foregoing data lies in the fact that the ation of nitrogen in cultivated soils is limited, among other things, by e energy available, that is, by the quantity of readily decomposable ganic residues. An indication as to the extent of these is given by the ount of humus present; nevertheless, this must remain an indication rely, for most of the humus is too inert to serve as a source of energy *Azotobacter*. From the data at present available different investitors have estimated the quantity of nitrogen fixed by *Azotobacter* 6.8 kg. to 18 kg. (15 to 40 pounds) per acre, per annum. Assuming vorable conditions for fixation, so that 500 g. (1 pound) of nitrogen uld be fixed for every 125 g.(100 pounds) of carbohydrate consumed, would still take an equivalent of 680 kg. to 1,814 kg. (1,500 to 4,000 unds) of sugar to produce this quantity of combined nitrogen. It may noted in this connection that *Azotobacter* have been demonstrated live in symbiosis with algæ, obtaining thereby the necessary energy their activities. This may explain, perhaps, the remarkable facts served by Headden in Colorado, relating to the accumulation of such ormous quantities of nitrate in the soil, as to destroy all vegetation. some instances the nitrates were found to be present to the extent of ,718 kg. (100 tons), or more (per acre), to a depth of a few inches. If e accumulation of combined nitrogen was due to *Azotobacter*, as is imed by Headden, and the bacterial residues oxidized by nitrifying cteria to nitrates, it is difficult to account for the source of the 1,000 2,000 tons of carbohydrates necessarily used up in the process of ation, unless it could be proved that the energy was furnished by gæ.

Symbiotic Fixation

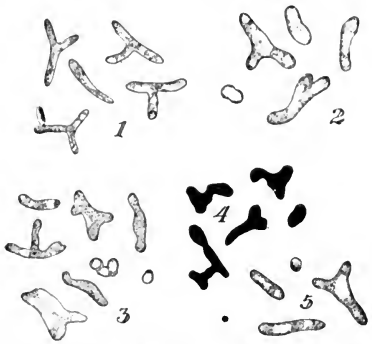
Historical.—Empirical observations extending well back into cient agriculture have led to the recognition of the soil-enriching

qualities of certain crops of the legume family. Columella mentions the fact that many Roman farmers regarded beans as possessing these qualities, but does not accept this belief for himself. On the other hand, he points out that luzerne (alfalfa), lupins and vetches improve the land and act as manure. He points out, also, that it was the practice of Roman farmers to plow under lupines in order to enrich the soil. In the centuries following the fall of Rome the use of legumes for soil improvement persisted to some extent in Italy, France and other countries; yet the practice was not followed consistently and the fertility of European soils was declining for lack of available nitrogen and, to a large extent, also of phosphoric acid. The more general introduction of clover into Germany and England in the eighteenth century helped to restore the fertility of many farms, and led, ultimately, to the recognition of the peculiar place held by legumes in the maintenance of soil fertility. But while practical farmers knew of the soil-enriching power of legumes, and while they retained their belief in it even when it seemed contrary to scientific authority, they did not know the secret of this power. It remained for Hellriegel and Wilfarth to demonstrate in 1886, and more fully in 1888, that this power, already hinted at by the investigations of others, is the resultant of the combined activities of the plants and of bacteria that enter their roots, and produce there the well-known nodules or tubercles. They showed in no uncertain manner that legumes can improve the soil only in so far as they add nitrogen to it with the aid of the bacteria in the tubercles; in other words, legumes were shown to enter into a symbiotic relationship with certain bacteria and to acquire, thereby, the ability to fix atmospheric nitrogen.

The presence of tubercles on the roots of leguminous plants was first recorded by Malpighi in 1687. He regarded them as root galls. The botanists who studied them in the first half of the nineteenth century classified them as modifications of normal roots or as pathological processes. In 1866 the Russian botanist Woronin found that the tubercles were filled with minute bodies resembling bacteria and concluded that they were pathological outgrowths. Some years later Frank, in 1879, not only showed that tubercles are almost invariably present on the roots of legumes, but that their formation may be prevented by sterilizing the soil. Frank was thus in possession of facts that might have revealed to him the true nature of the root-tubercles

Hwever, he later modified his belief in the origin of tubercles as due t outside infection, and accepted the interpretation of his pupil Brunchhorst who claimed that the bacteria-like bodies in the tubercles vre merely reserve food materials. Because of their resemblance to bcteria Brunchhorst named them bacteroids.

The studies of Marshall Ward, published in 1887, proved not merely tat tubercle formation is due to outside infection, but that such infection may be brought about at will by placing the roots of young plants in contact with pieces of old tubercles. Hellriegel in his preliminary cmmunication of 1886 also showed that outside infection is necessary f the production of tubercles, and called attention to the true func-



F. 121.—*Ps. radicicola.* 1, From *Melilotus alba;* 2 and 3, from *Medicago sativa.* 4, from *Vicia villosa.* (*After Harrison and Barlow from Lipman.*)

tn of the latter as laboratories wherein nitrogen compounds are nufactured out of clementary nitrogen. The true worth of Hellrgel's investigations was brought out more clearly in another paper t.t he published jointly with Wilfarth in 1888. The authors showed t.t in sterilized soils legumes behaved precisely like non-legumes, and dd ultimately of nitrogen hunger when not provided with nitrates or oer suitable nitrogen compounds. On the other hand, when the srilized soil was later infected with a few drops of leachings from fresh sl that had supported a normal growth of legumes, the starving plants rovered and grew vigorously. Under the same conditions nonlumes did not recover. The recovery of the starving legumes was fnd to be coincident with the formation of tubercles.

Hellriegel and Wilfarth's studies were soon confirmed by the inves tigations of others. Wigand showed in 1887 that the tubercles cor tained within them were true bacteria. In the following year Beyerinc reported the successful isolation of these bacteria on artificial medi and named the organism *B. radicicola* (Fig. 121). Prazmowski als isolated pure cultures of *Ps. radicicola*, and followed the entrance the organisms into the root hairs of young plants, their passage throug the cell-walls, and their transformation into bacteroids. These fac were all confirmed by other investigators, and it was further shown b Schloesing and Laurent that properly inoculated legumes not only ca grow in soils devoid of combined nitrogen, but that when growing such soils in a confined atmosphere they decrease the quantity nitrogen gas surrounding them by transforming it into nitrogen con pounds. It was, therefore, made clear by these investigations, and t

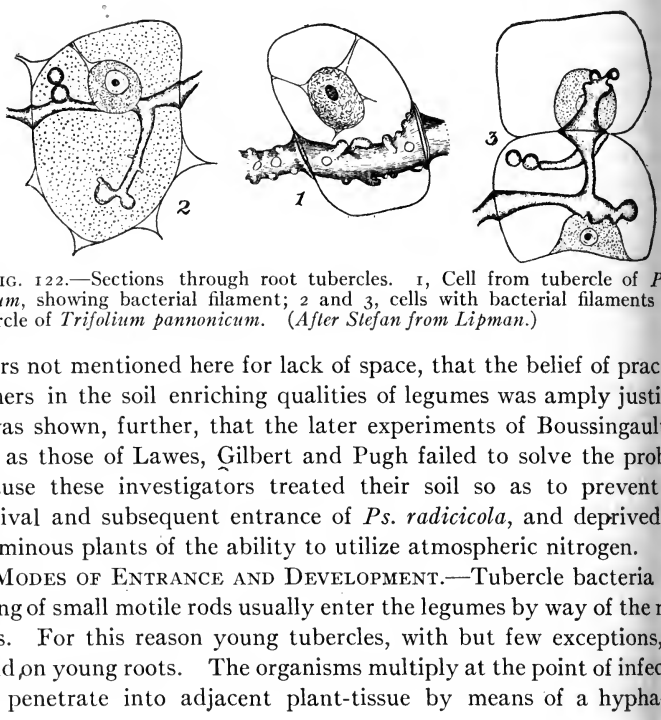


FIG. 122.—Sections through root tubercles. 1, Cell from tubercle of *Pisi sativum*, showing bacterial filament; 2 and 3, cells with bacterial filaments fr tubercle of *Trifolium pannonicum*. (*After Stefan from Lipman.*)

others not mentioned here for lack of space, that the belief of practic farmers in the soil enriching qualities of legumes was amply justifie It was shown, further, that the later experiments of Boussingault, well as those of Lawes, Gilbert and Pugh failed to solve the proble because these investigators treated their soil so as to prevent t survival and subsequent entrance of *Ps. radicicola*, and deprived t leguminous plants of the ability to utilize atmospheric nitrogen.

MODES OF ENTRANCE AND DEVELOPMENT.—Tubercle bacteria co sisting of small motile rods usually enter the legumes by way of the ro hairs. For this reason young tubercles, with but few exceptions, a found on young roots. The organisms multiply at the point of infecti and penetrate into adjacent plant-tissue by means of a hypha-li

ollow thread or tube that seems to consist of a gelatinous material Fig. 122). The tubes branch out as they pass from cell to cell and carry he invading organisms with them. The bacteria which may be readily etected within the tubes and cells are the involution forms of *Ps. adicicola* and assume various irregular shapes. They are designated s bacteroids. Stefan has suggested that bacteroids may be produced ithin the tubes and, possibly, from the buds or swellings that appear n the tubes. While still young, the bacteroids are capable of dividing, ut as they grow they swell up and finally degenerate.

Resistance, Immunity and Physiological Efficiency.—The nvasion of legumes by *Ps. radicicola* and the acquisition by the plant, hanks to this invasion, of the power to fix elementary nitrogen are cited s a case of symbiosis. However, some writers would regard the presnce of *Ps. radicicola* in legume roots as a case of parasitism. According o them symbiosis presupposes the living together of two organisms ith resulting benefit to both. In the present instance, however, onditions may arise when the host plant is injured, rather than benefited; and similarly, conditions may arise when the invading bacteria re suppressed by the plants. Making due allowance for the objections raised we still find, nevertheless, that in the broad relation of he two groups of organisms there is an apparent benefit to both plants nd bacteria. The former gain an adequate supply of nitrogen and he latter a supply of carbohydrates and of mineral salts.

A more detailed study of this relation shows that the plants resist he entrance of bacteria. When an abundance of available nitrogen ompounds is supplied tubercle formation may be largely or wholly uppressed. In that case the plants secure their nitrogen from the soil nd are not only independent of the bacteria, but are strong enough to esist their entrance. It is further claimed by Hiltner that tubercle acteria differ in their virulence, and that the more virulent the organms, the more readily will they penetrate the root tissue. Moreover, e believes that when a plant is invaded by organisms of any degree of irulence, the host plant becomes immune to a large extent and can keep ut all but the most virulent bacteria. The use of the term virulence, this connection, has been objected to, since it is borrowed from nimal pathology and is likely to be misleading. It is better to employ he term *physiological efficiency* as implying not only a more prounced ability to enter the plant roots, but also to fix atmospheric

nitrogen. It is conceivable that strains of *Ps. radicicola* may be developed that would grow rapidly and yet possess but a feeble nitrogen-fixing power. In other words, they would possess a high vegetative power and a low physiological efficiency.

MECHANISM OF FIXATION.—It is generally believed that the fixation of nitrogen is accomplished by the bacteria within the tubercles. The claim, at one time, advanced by Stoklasa, that the fixation is accomplished by the plants themselves with the aid of enzymes produced by the bacteria in their roots, has been disproved. It is known that the period of active nitrogen assimilation by the plants coincides with the appearance of the bacteroids in the tubercles, and it is supposed that the microörganisms fashion nitrogen compounds out of atmospheric nitrogen by using the carbohydrates and organic acids in the plant juices as a source of energy. The plants then seem to utilize the soluble nitrogen compounds that pass out of the bacterial cells. It is further supposed that bacteroid formation is an attempt on the part of the microörganisms to adjust themselves to the drain caused by the activities of the host plant.

VARIATIONS AND SPECIALIZATION.—Apparent differences in bacteria from different legumes were noted by Hellriegel. Some of his experiments indicated that bacteria from clovers could not produce tubercles on lupines and serradella. Analogous differences were found by Nobbe and his associates, nevertheless they were finally led to conclude that the root invasion of legumes is caused by a single species. However, continued association with any particular legume accomplishes in the end a certain modification, or specialization, as it were, of the microörganisms, and they were then no longer able to invade the roots of other legumes. Later, Hiltner and Störmer have been led to modify this view and have arranged the tubercle bacteria in two groups, possessing, according to them, well-defined morphological and physiological differences. One of these groups is included under the species "*Rhizobium radicicola*" and the other under "*Rhizobium beyerinckii*." The former comprises the organisms from lupines, serradella and soy beans while the latter comprises all of the others.

RELATION TO ENVIRONMENT.—Nitrogen fixation by leguminous vegetation is readily influenced by soil conditions, particularly the supply of lime and of other basic substances; the supply of organic matter and the aeration of the soil. As to the first of these it is well

hown that all legumes, with the exception of lupines and serradella, e stimulated in their growth by generous applications of lime.

FIG. 123.—These two pea plants were grown in clean quartz sand to which had en added small quantities of all the necessary elements of plant food *except trogen.* The conditions were exactly identical except that plant A was without ot nodules (see Fig. 124) and plant B had numerous nodules well developed (see g. 125). (*Mich. Exp. Station.*)

he top dressing of lawns with lime, marl or wood ashes encourages ie appearance of white clover; an adequate supply of lime makes

possible the successful growing of alfalfa in almost any soil, while the leguminous vegetation of limestone soils is proverbially vigorous. The favorable influence of lime is due to the direct action on the plants as well as on the bacteria in the soil. Similarly, the tubercle bacteria are favorably affected in their survival and multiplication by an abundant supply of organic matter. On the other hand, acid soils or those deficient in humus and inadequately aerated are but ill suited to the activities of *Ps. radicicola*.

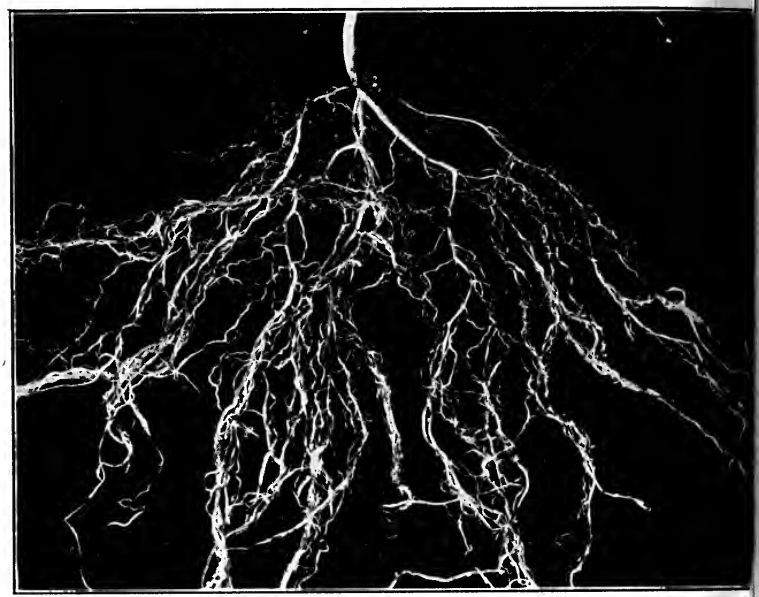


FIG. 124.—Roots of Plant A without nodules (Fig. 123).

SOIL INOCULATION*

By soil inoculation is now understood the adoption of some artificial method for supplying suitable quantities of nitrogen-fixing organisms to soils deficient in these types. The first attempts at soil inoculation were made in 1886 by Hellriegel and Wilfarth during the

* Prepared by S. F. Edwards.

course of their studies on the cause of nitrogen accumulation by legumes. They found that when leguminous plants were grown in sterile sand, nodules were formed on the roots only after the addition of a small portion of aqueous extract of fertile soil, or an extract of crushed nodules, or in some cases (lupines and seradella) by soil itself from a field on which these crops had been grown. The first successful artificial production of nodules by the aid of pure cultures was made

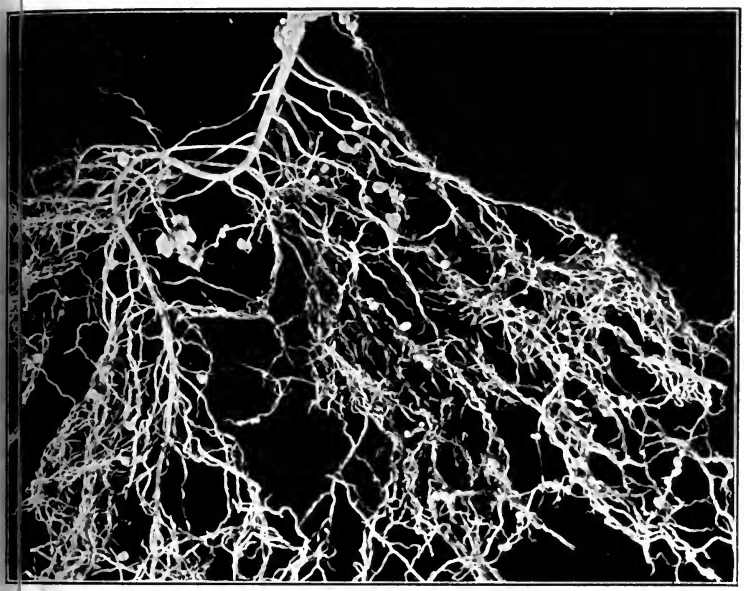


FIG. 125.—Roots of plant B with nodules (Fig. 123).

in 1889 by Prazmowski in the course of studies on the method of entrance of the organism to the root hairs of the host plant.

The first inoculation experiments in a large way were those made in 1897 at the Moor Soil Experiment Station, Bremen, Germany, where earth taken from fields that had borne luxuriant crops of various legumes was scattered over reclaimed heath or swamp soils upon which legumes had not previously grown, with the result that in every instance the yield on the inoculated portions of land was greater than on the

uninoculated plots. After such favorable results, it was but a natura step to try the effect of similar applications of soil rich in the nodule forming bacteria to ordinary cultivated soils of varying character While results in some cases were eminently satisfactory in others ther was no increase in the vigor or amount of the crop as a result of th inoculation.

METHODS OF SOIL INOCULATION.—From these early experimenta results there evolved two general methods of inoculation, namely, th application of soil from an already inoculated field, and the applicatio of pure cultures of the nodule-forming bacteria to the seed befor sowing.

Inoculation with Legume-earth.—The use of soil as inoculatin material was tried by various experiment stations of the United State with results not varying widely from those secured in the pione experimental work at Bremen. It was found in general that th commonly grown crops, such as the common clovers, peas and bean made little or no increase as a result of inoculation with old legume-soi With new crops, however, such as alfalfa and soy beans when they we first introduced, it was found impossible in many places to secure successful stand until the fields on which these crops were to be grow had received a top-dressing of soil from land that had already grow the crop in question; and it became a common practice to inocula soil in this manner before seeding with these new crops. It was ear observed, however, that this method of soil transfer for inoculatic purposes was not an unmixed benefit. Aside from the expense ar difficulty of handling and transportation of soil, fungus and bacteri diseases, not only of legumes but of other crops, as well as the see of noxious weeds, were transmitted from one field to another and ev from one section of country to another. It was to avoid this difficul that the preparation of pure cultures was introduced.

Inoculation with Pure Cultures. Nitragin.—The first pure cultu method was launched in 1896 by Nobbe and Hiltner, German inves gators, who prepared cultures of the legume bacteria on nutrient gelat and arranged with a firm of manufacturing chemists to place them the market under the trade name of *Nitragin.*

Dried Cultures.—In the United States the matter of pure cultu was first taken up by the Department of Agriculture about 19 Cultures of the nodule-forming bacteria were cultivated in nitroge

ee culture media, dried on cotton and distributed to farmers with a nall package of salts from which a culture solution was to be made y the farmer and applied to the seed. This method gave poor results, iefly because the bacteria could not withstand the drying on cotton. fterward the cultures were sent in a liquid condition with somewhat ore satisfactory results. The dry cotton cultures were exploited r a time by a commercial firm under the name of *Nitro-culture*, and mewhat similar cultures were placed on the market in England under e name of *Nitro-bacterine*. Cultures of both kinds, however, were own to be valueless, both by microbiological and by planting tests.

Cultures on Agar.—Very satisfactory results were secured from the e of pure cultures at the Ontario Agricultural College, Guelph, where arrison and Barlow, in 1905, originated the method of growing the cteria on a nitrogen-poor agar medium. By this method, the farmer s simply to apply the bacteria to the seed just before sowing. These ltures, used on all the common legumes, sown in all kinds of soil, ve favorable results in 65 per cent of cases in trials extending over a riod of ten years. Similar agar cultures are now prepared by commercial firms who have adopted the method of Harrison and Barlow, d also by some of the U. S. Agricultural Experiment Stations.

Importance of Inoculation.—Inoculation with pure cultures affords e farmer a rapid, easy, and cheap method of supplying the bacteria sential for getting a successful stand of any legumes. Failure to secure benefit from this method of inoculation may usually be attributed to nsuitable soil conditions rather than any inherent failing in the cultures used. No method of inoculation will compensate for poor ysical or chemical condition of the soil itself. The principle of using tificial cultures to be applied with the seed is sound, and if the cultures contain large numbers of virile bacteria, there is little reason hy they should not prove of benefit when used under soil conditions at would seem to need inoculation.

Azotobacter Cultures.—Some experimental work has been done in e use of cultures of Azotobacter for soil inoculation. The results are ntradictory, and more work needs to be done to prove the value such cultures.

CHAPTER IV

CHANGES IN INORGANIC CONSTITUENTS

Weathering Process

Origin and Formation of Soil.—Rock surfaces exposed to the action of rain, sunshine and frost lose their fresh appearance, become pitted and uneven, and gradually crumble into larger and smaller fragments. In the course of time the layer of disintegrated material becomes deeper and its constituent particles smaller—thanks to the uninterrupted process of subdivision. Finally, lichens, algæ and bacteria make their appearance, the organic débris accumulates, and higher plants begin to find a suitable environment for their development. The rock has changed into soil.

Influence of Biological Factors.—Soil-formation is not entirely a mechanical or chemical process. Even before the layer of weathered rock acquires any appreciable depth microscopical and macroscopical forms of life gain a foothold on the uneven surface. With the aid of sunlight they build organic compounds and make use of the combined or elementary nitrogen of the atmosphere. Their life activities result in the production of carbon dioxide and of varying organic and inorganic acids which in their turn react with the constituents of the rock particles. In this manner the biological activities become of utmost moment in the transformation and migration of mineral substances in nature. They assume an important rôle in the circulation of calcium and magnesium, with the accompanying phenomena that find most striking expression in the formation of caves and canyons in limestone strata. They assume a no less important rôle in the circulation of sulphur, in the accumulation and removal of available potash compounds in the soil, as well as in the transformation of phosphorus and its migration from inorganic to organic compounds.

Lime and Magnesia

Removal and Regeneration of Carbonates.—Lime and magnesia are present in soils in different combinations. They may occur

a silicates, carbonates, phosphates, humates, sulphates, etc. In humid climates the carbonates are being continually removed from weathered rock material, as is plainly shown by the composition of drainage waters. The losses become much greater in cultivated soils—thanks to the humus and the microörganisms present in them. The absolute amounts lost from year to year will depend on the proportion of lime and magnesia in the soil, the mechanical composition of the latter, its content of humus and the methods of tillage and fertilization. According to Hall the soils of the experiment fields at Rothamsted, containing about 3 per cent of calcium carbonate, are losing lime at the rate of 362 kg. to 453 kg. (800 to 1,000 pounds) per acre annually. In certain sections of Scotland where liming has been practised for a long time the farmers estimate the loss of lime from the land at 6 bushels per acre, annually; that is, approximately at the rate of 226 kg. to 272 kg. (500 to 600 pounds). In New Jersey, New York, Pennsylvania and other eastern states farmers who use lime more or less regularly apply 1 ton of it at the beginning of each five-year rotation. This would provide for an annual loss of 181 kg. (400 pounds) per acre. The loss of lime and magnesia is increased under intensive methods of agriculture. When animal manures and green manures are employed, microbial activities are stimulated, the production of carbon dioxide is encouraged and the loss of the soluble calcium bicarbonate made greater. The removal of lime is hastened even to a more striking extent when ammonium salts are applied to the land. The resulting nitrification and loss of lime are illustrated by the following equation:

$$(NH_4)_2SO_4 + 2CaCO_3 + 4O_2 = Ca(NO_3)_2 + CaSO_4 + 4H_2O + 2CO_2$$

As was already indicated, the loss of calcium and magnesium carbonate from the soil is effected largely through the activities of bacteria and of other microörganisms. At the same time microörganic life is responsible for the restoration of varying amounts of carbonates. It has been demonstrated that, in the weathering of the complex silicates, carbonates and silicic acid may be formed in considerable quantities. In the presence of decaying organic matter and the consequent evolution of carbon dioxide the formation of carbonates from silicates may be extensive enough to balance the losses. Similarly, calcium carbonate may be formed in the soil from humates and from the calcium salts of simpler organic acids. They may be formed, also, through the activi-

ties of denitrifying and other reducing bacteria from the correspondin nitrates and sulphates. As pointed out by Nadson ammonium ca bonate produced in the decomposition of protein compounds may rea with calcium sulphate as follows:

$$(NH_4)_2CO_3 + CaSO_4 = CaCO_3 + (NH_4)_2SO_4$$

Moreover, calcium sulphate may be reduced to sulphide and may rea with carbon dioxide as follows:

$$CaS + CO_2 + H_2O = CaCO_3 + H_2S$$

Magnesium would be subject to similar reactions and Nadson h observed the formation of a mixture of calcium and magnesium ca bonates (corresponding to dolomite in composition) in media inoculat with a pure culture of *B.* (*Proteus*) *vulgaris*.

Lime as a Base.—The carbon dioxide generated in vast amounts the life processes of most soil bacteria, the nitrous and nitric aci formed by the nitro-bacteria, the sulphuric acid produced in t oxidation of hydrogen sulphide and of sulphur by the so-called sulph bacteria, and the great variety of organic acids formed in the deco position of carbohydrates, fats and proteins all react with basic su stances in the soil. Of these basic substances calcium carbonate is l far the most prominent. Combining with the different acids maintains a favorable reaction for microörganic life in the soil.

The calcium salts thus formed are more or less soluble. In t manner enormous amounts of lime are annually carried to the oce as bicarbonates, and to an appreciable extent also as nitrate a sulphate. Thus soil bacteria help to furnish shell fish and other for of marine life, the material necessary for the building of their skeleto In the course of ages the latter become a portion of the solid land and coral reefs, chalk cliffs and marl beds offer to microörganisms a n opportunity to start calcium carbonate on its migrations.

Effect of Calcium and Magnesium Compounds on Bacteri Activities.—Being basic in character calcium and magnesium c bonates are of great service in maintaining a suitable reaction in t soil. But somewhat apart from this service calcium and magnesi compounds seem to be particularly important for the growth of cert organisms. It has already been observed by Winogradski and On lianski that magnesium carbonate is especially useful in facilitating t

plation and culture of nitrate bacteria. Heinze and others have ted the favorable action of calcium carbonate on the growth of *otobacter*, while the beneficial influence of calcium carbonate and sul- ate on the development of *Ps. radicicola* has been repeatedly observed different investigators.

Phosphorus

Availability of Phosphates.—Phosphorus exists in the soil largely the form of phosphates of calcium, magnesium, iron and aluminum. small portion of it occurs in organic combination in lecithin, phytin d other compounds. The soil phosphates possess a very slight degree solubility and often fail to become available rapidly enough to meet e demands of the growing crop. Fortunately the presence of rbon dioxide generated from decaying organic matter hastens the lution of the inert phosphates, thus:

$$Ca_3(PO_4)_2 + 2CO_2 + 2H_2O = Ca_2H_2(PO_4)_2 + Ca(HCO_3)_2$$

r this reason a maximum supply of available phosphates may be cured by plants in the presence of readily decomposable organic atter.

Apart from carbon dioxide as a means for making available inert osphates, bacteria produce organic and inorganic acids that are of rect service. The influence of nitrous, nitric and sulphuric acids, all them products of bacterial activity, is undoubtedly of some im- rtance. The influence of lactic, acetic and butyric acids, as well as the more complex humic acids, must be of considerable moment. r instance, in the decomposition of bone meal by *B. mycoides*, oklasa found that 23 per cent of the phosphoric acid had become luble, whereas in similar uninoculated portions of bone meal only 3 r cent of soluble phosphoric acid was found. The significance of ganic acids produced by microörganisms is brought out even more rongly in the loss of phosphates from acid soils.

In so far as the organic phosphorus compounds are concerned bac- rial activities are important in that the processes of decay restore the osphorus to circulation. Hence, it will be seen that microörganisms e directly concerned in the migration of phosphorus from the soil to e plant and from the plant back to the soil.

Relation of Phosphorus to Decay and Nitrogen Fixation.— st as bacteria influence the transformation of phosphorus compounds

in the soil, so phosphorus itself affects the growth and activities o bacteria. As one of the essential constituents of living cells it reac on the growth of microörganisms and influences species relationship There are undoubtedly species whose phosphorus requirement is greate than that of other species. Indeed, conditions may arise that favor th rapid assimilation of soluble phosphates by bacteria. In that case th microörganisms would act as competitors to the higher plants. Amon the species favorably affected by an abundant supply of phosphat *Azotobacter* is quite prominent. Hence nitrogen fixation is in a mea ure dependent upon a proper supply of phosphorus compounds.

Sulphur

Sulphur Compounds in the Soil.—Sulphur occurs in the soil i the form of sulphates and in that of organic compounds. In il aerated soils the reduction products of sulphates; viz., sulphites, su phides and even elementary sulphur may be present in small amoun as a transition stage. According to Berthelot and André the prote compounds of the soil humus are quantitatively more important tha the sulphates. However, this is not true of arid and semi-arid soi in which sulphates represent a larger store of combined sulphur tha is contained in organic substances.

Sulphur Bacteria.—In the decomposition of protein compoun with a limited supply of air, hydrogen sulphide and mercaptans a evolved. The quantities of hydrogen sulphide produced may l large enough to become perceptible to the sense of smell, as happens the putrefaction of eggs. At the bottom of seas, rivers, lakes ar ponds (in canals, ditches, swamps, etc.) as well as in finer-grained soi the production of hydrogen sulphide goes on almost uninterrupted owing to the activities of a great variety of bacteria. The hydroge sulphide thus generated serves as a source of energy to a group organisms known as sulphur bacteria. The oxidation of the h drogen sulphide by these bacteria may be expressed by the followi equations:

$$2H_2S + O_2 = 2H_2O + S_2$$
$$S_2 + 2O_2 = 2SO_2$$

The sulphur dioxide produced is further changed into sulphuric ac in the presence of oyxgen and water. In its turn the acid reacts wi

ome base, usually calcium carbonate, resulting in the formation of alcium sulphate. Thus:

$$SO_2 + O + H_2O = H_2SO_4$$
$$H_2SO_4 + CaCO_3 = CaSO_4 + H_2O + CO_2$$

We owe much of our knowledge concerning the sulphur bacteria to Vinogradski. This investigator showed that in places where hydrogen ulphide is generated in considerable quantities sulphur bacteria grow igorously and accumulate granules of sulphur within their cells. When the cells containing sulphur granules are removed to suitable nedia, in which no hydrogen sulphide is present, the sulphur seems o be gradually oxidized and disappears and the bacteria finally die of tarvation. Thanks to the sulphur bacteria, the higher plants are nabled to utilize again the sulphur once locked up in plant and ani-nal tissues, and liberated thence by decay bacteria. The circulation f sulphur is thus made possible and the cycle is completed when the ulphates are again used by plants to build protein compounds. It nay also be noted in this connection that "*Thiobacillus denitrificans*," escribed by Beyerinck, may also oxidize elementary sulphur. In his case, however, the oxygen is derived from nitrates instead of the tmosphere. Thus:

$$6KNO_3 + 5S + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 3N_2$$

Sulphofication.—Lint has found that under optimum temperature nd moisture conditions, sulphur applied at the rate of 600 pounds er acre was almost completely oxidized within ten weeks. Boullanger nd Dugardin in explaining the fertilizing action of sulphur on the asis of its effect on the supply of available nitrogen found that am-monification was increased by small amounts of sulphur, nitrogen-ixation was not affected and nitrification was depressed. It has been ointed out by Kossovitch, Brioux and Puerbet that the mechanism f sulphur fertilization is very complex and that the oxidation of free ulphur occurs entirely by bacterial and not by chemical means. Brown and Kellogg have recently advanced evidence to prove that soils have a definite sulphofying power which is determinable in the laboratory by a newly devised method. They claim that the process of sulphofication is mainly brought about by bacterial action, but

probably there is also a small production of sulphates in soils due to chemical action.

It has been observed that soils differentiated by various treatments, vary widely in sulphofying power, the presence of organic matter being responsible for an increase up to a certain point. Aeration and moisture must be optimum for favorable sulphofication while the addition of carbohydrates to soils depresses the process.

Sulphate Reduction.—The fact that sulphates may be reduced to sulphides in the presence of organic matter has been known for many years. In compost heaps, and at the bottom of seas, lakes and rivers, the reduction of calcium sulphate is of common occurrence. Similarly, ferrous sulphate may be reduced in water-logged soils and in swamps and may give rise to deposits of bog iron. But while sulphate reduction is of common occurrence in certain localities, it has been shown by Beyerinck and also by van Delden, that the reduction can be accomplished in artificial media by specific microörganisms. Two species isolated by these investigators have been named *Sp. desulphuricans* and *Msp. æstuarii*. When grown under anaerobic conditions in culture media supplied with combined nitrogen and organic nutrients these organisms were found capable of reducing sulphates. The oxygen withdrawn from the sulphates was used for the oxidation of organic matter in a manner analogous to that in nitrate reduction where the oxygen is derived from the nitrates. Apart from the two organisms that cause the specific reactions just noted, there are many common soil bacteria that may be responsible for sulphate reduction in a less direct manner. Nadson has observed that when the supply of oxygen is limited calcium sulphate may be reduced to sulphide by *B. mycoides* and by *B.* (*Proteus*) *vulgaris*. The calcium sulphide according to him may react with carbon dioxide and water, giving rise to the formation of hydrogen sulphide. Thus:

$$CaS + CO_2 + H_2O = CaCO_3 + H_2S$$

The hydrogen sulphide derived from sulphates or from proteins becomes a source of energy to the sulphur bacteria as already noted in the preceding pages.

Potassium

The Transformation of Potassium Compounds in the Soil.—Potassium occurs in the soil largely in the form of silicate minerals.

Smaller amounts occur as nitrate, carbonate and in organic compounds. The portion present as silicates is often very large in clay-loam soils, amounting not infrequently to 22,679 kg. to 34,019 kg. (50,000 to 75,000 pounds) per acre-foot. Unfortunately for the farmer, the growing crops fail, in many cases, to secure sufficient quantities of available potash for their rapid development, notwithstanding these enormous stores of potassium compounds. However, when sufficient quantities of readily fermentable organic matter are present and the generation of carbon dioxide is rapid the silicates weather sufficiently fast to meet the demands of maximum harvests. The part played by carbon dioxide in the transformation of inert potash compounds may be illustrated by the following reaction:

$$Al_2K_2O\,6SiO_2 + CO_2 + 2H_2O = Al_2O_3\,2SiO_2\,2H_2O + K_2CO_3 + 4SiO_2$$

Under actual conditions it is the aim of the farmer to stimulate bacterial activities (and, therefore, the production of carbon dioxide) in his land by the use of animal manures or green manures and of commercial fertilizers. Apart from the influence of carbon dioxide available potash compounds may likewise be formed on account of nitric, sulphuric, acetic, lactic, butyric and other acids produced by different soil bacteria.

Other Mineral Constituents

Iron.—The investigations of Ehrenberg, Winogradski, Molisch, Adler, Ellis and others have accumulated a mass of data relating to the so-called iron bacteria. These organisms belong to the class of higher bacteria and recently forms, such as rod-shaped bacteria, have been isolated which have a marked ability to precipitate iron oxide out of solutions of iron salts. Winogradski believed that the reaction is a physiological one in that the microörganisms oxidize ferrous to ferric compounds, and utilize for their growth the energy thus made available. The investigations of Molisch, Adler and Ellis show, however, that the iron bacteria can exist very well without iron compounds; and that the precipitation of iron oxide is due to mechanical rather than chemical influences. But whether physiological or mechanical the influence of the microörganisms is felt in the formation of bog iron, and in the filling up of iron pipes; in the latter instance much annoyance is occasionally experienced by those in charge of municipal water supplies.

Compounds of iron are of considerable significance in the l processes of many bacterial species. For instance, it was shown Lipman and after him by Koch, that *Azotobacter* will not develop in c ture media devoid of iron compounds. In field practice small applic tions of ferrous sulphate often seem to exert a favorable effect on cr growth. and there is reason to suspect that soil-microbial activities a of some moment in bringing about the results noted.

ALUMINUM, MANGANESE, COPPER.—Weathering processes and t relation of carbon dioxide to these processes have already been d cussed in connection with calcium and potassium compounds. T great extent aluminum is affected by these reactions, for in the decom sition of feldspar, kaolinite is one of the important products form Hence, bacteria become a factor of considerable importance in the forn tion of hydrated silicates of aluminum, at least, in the presence organic matter. Moreover, it is recognized in the ceramic indust that after it is dug clay must undergo ripening in order to be suita for certain purposes. The ripening process involves the activities bacteria. Unfortunately very little is known about the reactions t occur in the ripening of clay.

As to manganese and copper there is scarcely any experimental e dence available as to the part played by their compounds in the s particularly in so far as they affect microörganic life. To some exte it is known that where Bordeaux mixture has been employed for spr ing potatoes, cranberries, fruit trees, etc., plant growth is subsequer stimulated to a striking extent. In view of the very slight quantitie copper that are actually added to the soil by these sprays, it is poss that the effects noted are caused by stimulated or changed micro activities. This view finds some support in the influence exerted copper sulphate on the growth of algæ in lakes, ponds, and shal streams.

It has also been reported that the decomposition of complex silic has been effected from powdered minerals by nitrite bacteria.

ANTAGONISM

A subject which bids fair to become a fertile source of investiga is the application of certain biochemical laws, as established by I and Osterhout in the animal and plant worlds respectively, to

ect of salts on the physiological efficiency of soil bacteria in pure and xed cultures, as well as in the soil. C. B. Lipman has advanced information concerning the antagonism between anions as related to rogen transformations in soils, with special reference to the reclamatn of alkali lands. Antagonism exists to a more or less marked ent between anions of alkali salts (as for example between NaCl ad Na_2SO_4, Na_2CO_3 and Na_2SO_4 and between NaCl and Na_2CO_3) ven the ammonifying or nitrifying powers of the soil are employed a criteria. The nitrogen-fixing flora, however, is not similarly ected, apparently offering greater resistance. The practical sugtion carried out of such data then, involves the addition of salts t the toxic salts already contained in a given soil, and thereby impving its ammonifying and nitrifying power.

DIVISION IV

MICROBIOLOGY OF MILK AND MILK PRODUCTS

CHAPTER I*

THE RELATION OF MICROORGANISMS TO MILK

IMPORTANCE OF MILK AS A FOOD

Fresh normal milk is one of the most important of human food It has a pleasant taste and aroma and is generally liked as a food drink; but unless properly cared for will not long remain in its norm condition. No article of human diet is more susceptible to undesi able changes, due to the delicate nature of the milk itself and to th conditions naturally surrounding its production and handling. Th injurious changes which commonly occur in milk are of two kind

ABSORBED TAINTS AND ODORS

Milk is very quickly affected by odors of any sort. The forei odor may be absorbed before the milk leaves the udder if the cow h eaten strong feeds, such as cabbage, onions, etc., or it may be absorb after the milk is drawn from the cow. If milk is exposed to a strong odor, such as silage or foul air, resulting from lack of ventil tion in the stable at milking time, these odors will be taken up by t milk with surprising rapidity. If placed in an ice chest with fre strawberries or pineapple, or foods like cabbage or turnips, the m will very quickly absorb the odor of these foods. The absorpti of any foreign odor gives to milk a decidedly disagreeable taste. Th is true even when the odor which is absorbed is pleasant in itself in the case of strawberries or pineapples. When the "off" flavors a due to absorption they are strongest at the outset and become l

* Prepared by W. A. Stocking with the exception of the paragraphs treating the acid-form bacteria, prepared by E. G. Hastings.

pronounced as the milk becomes older, especially if it is subjected to me method of aeration.

Changes Due to Microörganisms

While absorption of foreign odors is not uncommon, probably st of the undesirable flavors, found in milk when it reaches the nsumer, are caused not by absorption but by the growth of icroörganisms in the milk. In this class the changes are slight at st and increase with the age of the milk. Changes of this sort clude the common phenomena of souring and curdling, the so-lled sweet curdling, ropy or slimy milk, bitter flavors, gassy milk d a large variety of changes usually known as barny or cowy odors d flavors. If milk could be kept free from microörganisms, it ght be kept for some time without showing perceptible changes in pearance or taste. No other food product will undergo fermenta-n changes as rapidly as milk because it is an ideal culture medium r the growth of most kinds of microörganisms, especially bacteria d yeasts. Not only does milk contain the needed food elements but, ing in liquid form, they are easily available for the use of micro-ganisms. The proteins and milk sugar are most easily attacked d it is the breaking down of these which causes most of the changes i the milk.

Microbial Content of Milk

When we recognize the extreme ease with which milk undergoes cterial changes, we are not surprised to find that ordinary milk, en delivered to the consumer, contains relatively large numbers bacteria. The amount of care exercised in the production and ndling is a most important factor in determining the bacterial ntamination of milk. On this basis milk may be roughly divided o three classes.

Common Milk.—Age is one of the chief factors in determining e germ content of milk. We are, therefore, not surprised to find e milk in large cities having a much higher germ content than in aller cities and towns. The normal germ content of ordinary lk as it is found in the cities may be shown by the following bles.

BACTERIA IN BOSTON MILK*

Average taken from 2,394 Samples
From June to September

	Per cent
Below 100,000 bacteria per c.c.	42.0
Between 100,000 and 500,000 per c.c.	29.75
Between 500,000 and 1,000,000 per c.c.	9.75
Between 1,000,000 and 5,000,000 per c.c.	12.75
Above 5,000,000 per c.c.	5.0
Uncountable plates.	0.75

BACTERIAL COUNTS OF CHICAGO (RAW) MILK†

Date	Number of samples	Average count	Lowest count	Highest count
January, 1910	64	1,067,000	27,000	5,500,000
April, 1910	43	5,948,000	14,000	150,000,000
July, 1910	183	12,548,000	8,000	190,000,000

BACTERIA IN MILK OF CONNECTICUT CITIES‡

Bacterial count	Number of samples
Under 50,000	1,707
50,000–100,000	130
100,000–500,000	459
500,000–1,000,000	98
Over 1,000,000	73

These figures give the results of 2,467 samples collected in seventy-five differe towns in the State from October 1, 1908 to October 1, 1909.

Goler gives the average bacterial count for 1,057 samples of market milk collect in Rochester during the year 1909 as 446,099 per c.c. Of these samples 1.79 per ce were above 5,000,000 and 38.4 per cent below 100,000.

In Montclair, N. J., the average bacterial count for the year 1909 from sampl representing fifty-seven dairies was 53,000 per c.c.

In Ithaca, N. Y., 148 samples were taken for the year beginning April 1, 190 and ending March 31, 1910. The average bacterial count of these samples w 221,000.

The immense numbers of bacteria found in milk in the large citi are usually the result of the rapid growth of the *Bact. lactis acidi* grou resulting from the age of the milk and the temperature at which it h

* Data given by Hill and Slack.
† Data given by Tonney.
‡ Data given by Conn.

be kept. Such milk may also contain large numbers of those saprophytic organisms which occur in the atmosphere and about the stables and milk-house. The number of this group depends largely upon the sanitary conditions of production and the initial contamination. In ordinary milk organisms of the *Bact. lactis acidi* type will constitute a very large percentage of those present when the milk reaches the city even before it shows any perceptible signs of souring. During the past few years great progress has been made in the production of clean milk, and at present quite an important part of the general milk supply of our cities has a very much lower germ content than it had a few years ago.

Special Milks.—In this class may be considered those milks known as *Selected*, *Inspected*, or *Guaranteed*. As commonly used these terms mean milk which has been produced and handled with considerably more care than ordinary market milk but not with the extreme care required for *certified* milk. Guaranteed milk is produced by herds which have been shown by the tuberculin test to be free from tuberculosis. Considerable care is exercised in all the operations of handling the milk. The result is that these milks usually have a much lower germ content than the ordinary milk supply of the same city. Sometimes the germ content of such milk compares favorably with that of certified milk. These milks may contain various types of normal milk organisms but they should not contain any tubercle bacteria.

Certified Milk.—Certified milk means milk which has been produced according to the regulations of and under the supervision of a medical milk commission. The stables and cows are kept extremely clean. No dust is allowed in the stable at milking time. The cow's flanks and udder are washed just before milking, the milkers wear white suits and wash their hands before milking each cow. Small-top pails are used and the milk is cooled as soon as drawn from the cow. The extreme care exercised in the production and handling of this milk has a very marked effect on the number of bacteria found in it. The following counts are typical of certified milk.

BACTERIAL COUNTS OF CERTIFIED MILK IN DIFFERENT CITIES

Boston, Oct. 1, 1909 to Sept. 30, 1910*

Dairy number	Number samples	Average bacteria
1	17	5,794
2	13	4,176
3	30	6,825
4	12	1,475
5	7	2,294

New York City, Oct., 1909 to Sept., 1910†

Farm number	Average count
1	11,132
2	10,516
3	8,504
4	16,193
5	2,863
6	11,246
7	23,705
8	5,370
9	15,062
10	459

Chicago‡

Dairy number	Number counts	Average number bacte
1	51	5,612
2	60	4,078
3	43	6,502
4	17	2,553

Brooklyn

Moak gives the average of 321 counts for certified milk delivered in Broo n during the first six months of 1910 as 4,095 bacteria per c.c. The best average i n any one farm was 561 bacteria per c.c.

* Data given by Arms.
† Data given oy Park.
‡ Data by Heinemann.

Sources of Microörganisms in Milk

The sources from which bacteria get into the milk have been the subject of much investigation during the past few years, until now the chief sources of contamination are pretty well understood. These sources may be grouped in a general way under the following heads:

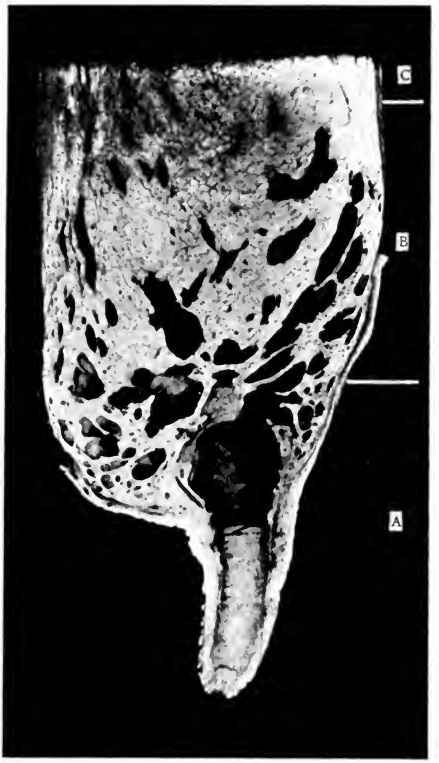


Fig. 126.—Vertical section of one quarter of udder showing teat, milk cistern, and larger milk ducts. (*After Ward and Hopkins.*)

Interior of the Cow's Udder. *Healthy Udders.*—Milk as it is secreted by the normal udder of a healthy cow is probably free from bacteria. It is very difficult, however, to obtain milk from the udder

which does not contain bacteria in greater or less numbers. This is due to the fact that immediately after secretion the milk becomes contaminated by bacteria which exist in the interior of the udder. Early investigators, notably de Freudenreich and Grotenfelt, believed that milk while in the udder was entirely free from microörganisms. Later investigations, however, by Moore, Ward, Bolley, Hall and others, have shown that the healthy udder normally contains bacteria in appreciable numbers. It has been found that bacteria are present even in the upper portions of the udder in the small milk passages leading from the secreting cells. These organisms, which normally exist in the milk passages of the udder, gain entrance through the orifice in the end of the teat where they find suitable conditions for growth and, once inside work up through the milk cistern to the larger milk ducts and finally though all parts of the udder (Fig. 126). The number of bacteria found in the udder varies widely in different cows as may be seen by the following figures:

Bacterial Content of Entire Milk of Different Cows

Cow No. 1	850 bacteria per c.c.
Cow No. 2	750 bacteria per c.c.
Cow No. 3	25 bacteria per c.c.
Cow No. 4	112 bacteria per c.c.
Cow No. 5	70 bacteria per c.c.
Cow No. 6	1,850 bacteria per c.c.

If portions of milk are taken at different intervals during the process of milking in such a way that all external contamination is prevented, it will be found that the first few streams of "fore-milk" contain many more organisms than the milk drawn later. After the first ten or twelve streams the number of organisms will decrease quite rapidly, normally becoming less and less until the final strippings, when there is usually a marked increase. This condition indicates that the larger number of organisms exist in the milk cistern and larger milk ducts in the lower part of the udder and are therefore removed during the early part of the milking. The increase at the end of the milking is probably due to the greater manipulation, resulting in dislodging some of the organisms which have adhered to the walls of the milk passages.

Not only does the number of organisms in different cows vary, but there is a marked difference in the different quarters of the same udder as shown by the following figures.

BACTERIA IN DIFFERENT QUARTERS OF COW'S UDDER*

	Right front quarter of udder		Left front quarter of udder		Right back quarter of udder		Left back quarter of udder	
	No. samples	Average per c.c.	No. samples	Average per c.c.	No. samples	Average per c.c.	No. samples	Average per c.c.
Herd of 1900–02.....	79	419	77	378	80	653	80	617
Herd of 1910–11.....	185	199	174	130	185	636	186	698
Herd of A. G. L.....	46	161	46	107	46	597	46	342
Averages.....	.....	249	.....	191	.....	635	.....	625

Average germ content per c.c. in 316 samples from herd of 1900–02..... 518
Average germ content per c.c. in 730 samples from herd of 1910–11..... 420
Average germ content per c.c. in 184 samples from herd of A. G. L..... 320
Average germ content per c.c. in 1,230 samples from 78 cows..... 428

The number of organisms normally found in the udder is much smaller than would be expected when we consider the fact that ideal conditions of food and temperature are provided there for bacterial growth. The relatively small number of organisms is perhaps due to some germicidal action existing in the udder. Attempts to increase the germ content in the udder by injecting cultures of different species of saprophytic bacteria have failed to produce a continued increase, the injected organisms usually decreasing very rapidly in numbers until they disappear at the end of a few days. From the standpoint of ordinary market milk, the number of bacteria found in the healthy udder is so small that it is of little commercial importance. In dairies where a very small germ content is desired, however, this source of infection must be taken into account and in certain cases individual cows, which normally have a high bacteria content in the udder, can be discarded to advantage.

It is evident that many species do not find the conditions in the udder suitable for their growth, since investigations have shown that comparatively few species exist for any length of time in the healthy udder. Certain types of micrococci are the predominating forms with occasional cultures of other species. The *Bact. lactis acidi* type does

* Harding and Wilson: Technical Bul. No. 27, N. Y. Agril. Exp. Sta., 1913.

not thrive in the udder. The types of organisms commonly found there do not seem to develop rapidly in the milk when it is held at low temperatures and fail to produce any appreciable changes in it during the normal life of market milk.

Diseased Udders.—If, however, the cow is suffering from disease in the udder, the bacterial condition may be quite different from that described above. In this case, the milk may be filled with the specific bacteria before it leaves the udder. In cases of inflammatory trouble or tuberculosis in the udder the milk may contain very large numbers of organisms, frequently many millions per c.c. at the time the milk is drawn.

Exterior of Cow's Body.—The nature of the cow's coat and the condition under which she is normally kept favor the accumulation of dust and bacteria upon her body. Unless special care is taken to keep the cow's body free from dirt, the organisms which fall into the milk from this source at milking time will constitute one of the most important sources of contamination. The importance of this source of contamination may be recognized when we see what large numbers of microörganisms may be carried by small particles of dust or a single individual cow hair.

The importance of this source of contamination depends very largely upon the conditions under which the cows are kept and the care exercised in cleaning just previous to milking. In many of the certified milk dairies this source of contamination is reduced to a minimum and has little effect upon the milk.

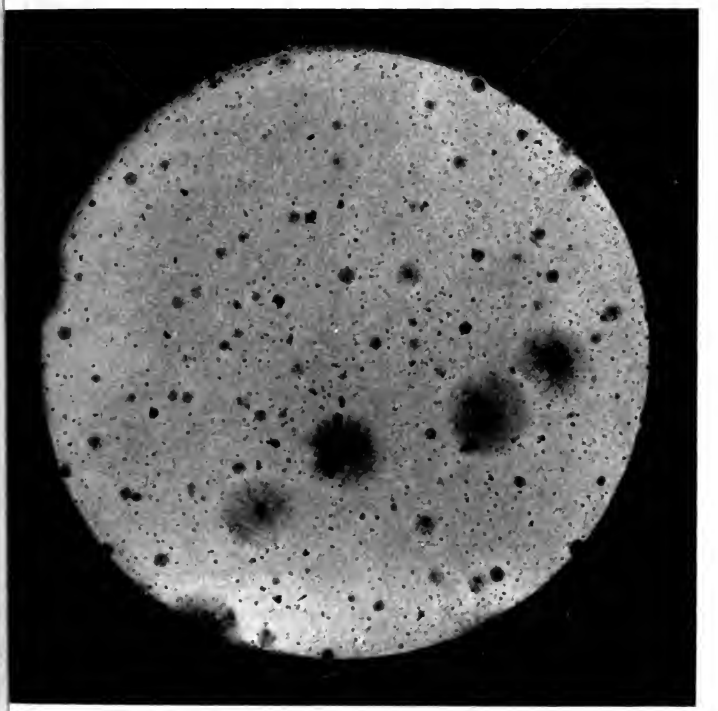
Atmosphere of Stable and Milk House.—The atmosphere of the stable is often a very important factor in determining the bacterial content of fresh milk. In sanitary dairies this factor is fully recognized and every effort is made to prevent the presence of dust in the atmosphere at the time of milking. The atmosphere is sometimes sprayed either with the hose or with steam in order to settle every particle of dust at milking time. In stables where the importance of this factor is not recognized and dust is allowed to exist in the atmosphere at milking time, the number of bacteria in the milk will be materially increased.

The Milker.—Not infrequently the milker himself is an important source of contamination. If his clothing and hands are dirty or if he brushes against the cow, the dust thus dislodged may carry into the milk large numbers of microörganisms. This is shown in the di

fence in the germ content of milk drawn by two men milking in the sme barn under identical conditions.

IFFERENCE IN NUMBER OF BACTERIA IN MILK DRAWN BY MEN IN SAME STABLE

	Number of milkings	Number of bacteria per c.c.
Mker No. 1	19	2,450
Mker No. 2	19	17,100



F. 127.—Colonies developing in agar plate held for ten seconds in position of milk pail after udder was brushed gently with the hand. (*Original.*)

THE UTENSILS.—If properly cared for, the dairy utensils should n add to the germ content of the milk. Not infrequently, however,

they are faulty in construction. In open seams and other places the milk may accumulate and not be thoroughly washed out. Usually when utensils of this sort are used, the methods for washing and sterilizing are not sufficient and bacteria multiply in large numbers in the cracks and crevices and contaminate each new lot of milk put into them. Sometimes the utensils which are properly constructed may

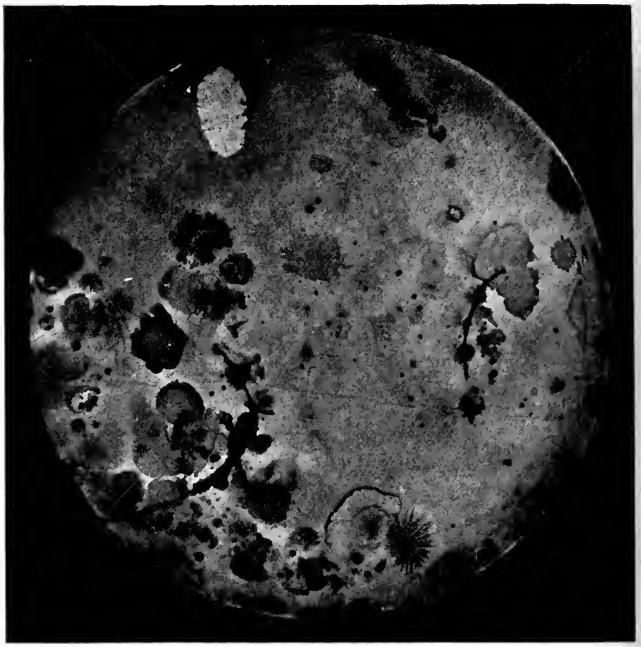
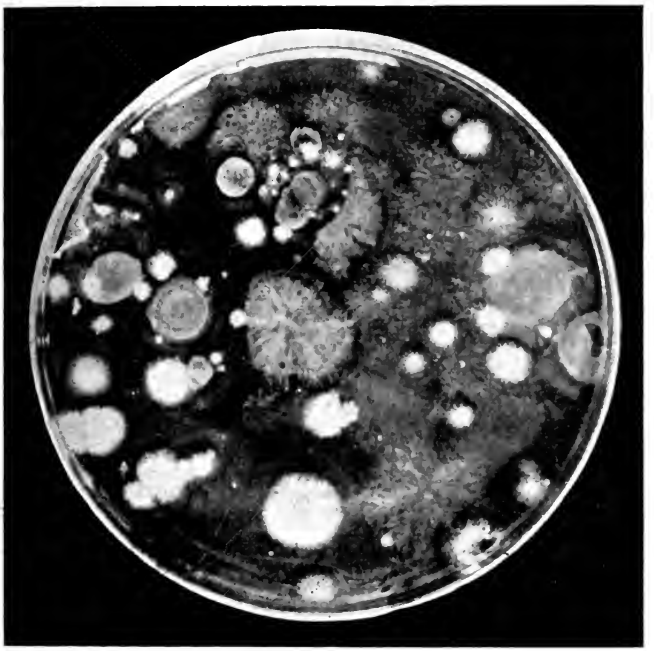


FIG. 128.—Colonies developing from cow-hairs planted in agar plate. (*Origina*

contaminate the milk because they have not been properly cleanse and sterilized. The use of steam is the most efficient means of ste ilizing all dairy utensils, but boiling water may give very satisfacto results if used at actual boiling temperature. If not used at the boili temperature some of the more resistant organisms will not be kill and will be left to inoculate the fresh milk. The ropy milk organis *B. lactis viscosus*, often remains in the utensils from day to day in th way.

WATER SUPPLY.—Sometimes the water used for washing the dairy tensils is a serious source of contamination. Serious epidemics of isease have been traced to this source where the utensils were washed ith water contaminated by typhoid or other disease organisms nd were not sufficiently sterilized to kill those remaining in the uten-ls. Such dairy troubles as ropy milk and gassy milk may be caused y the water used for washing purposes.



IG. 129.—Colonies developed from a bit of dust found in cow stable. Agar plate culture. (*Original.*)

METHODS OF PREVENTING CONTAMINATION OF MILK

INDIVIDUAL COWS.—Normally the number of microörganisms ound in the udder is not sufficient to be a serious source of contami-ation for market milk. There are, however, certain cows which ave a much higher germ content than others, and where a very low

count is desired in the milk, it may sometimes be advisable to eliminate such cows from the herd.

CARE OF THE COW'S BODY.—In order to reduce to the minimum the contamination from the cow's body, she should be kept as clean as possible. Dust should not be allowed to accumulate in her coat. It is well to keep the hair of the flank and udder clipped in order to prevent the accumulation of dust and also to facilitate the process of cleaning. The use of a damp cloth for wiping the flank and udder at milking time is a very efficient means of reducing this source of contamination. The beneficial effect of this method may be seen in the following table:

EFFECT OF WIPING UDDER AND FLANK WITH A DAMP CLOTH AS SHOWN BY BACTERIAL COUNTS OF MILK

Number of experiments	Date	Treatment	Bacteria per c.c.
1	Apr. 13	Not wiped	2,780
		Wiped	530
2	Apr. 15	Not wiped	1,310
		Wiped	310
3	Apr. 16	Not wiped	800
		Wiped	754
4	May 28	Not wiped	1,130
		Wiped	590

Even when considerable care is taken to clean the surface of the cow's body, there will still be some organisms which may fall into the pail at milking time. This number can be very materially lessened by reducing as far as possible the area through which dust can fall into the milk pail. This can be accomplished by the use of a milking pail with a small top.

VALUE OF SMALL TOP PAIL IN REDUCING GERM CONTENT OF MILK

Experiment	Kind of pail	Bacteria per c.c. of milk
No. 1	Open	15,500
	Small top	7,750
No. 2	Open	3,700
	Small top	1,100
No. 3	Open	30,000
	Small top	4,700

FIG. 130.—Some different styles of small top milking pails which are practical and efficient. (*Original.*)

AVOID DUST IN THE ATMOSPHERE.—Many of the necessary operations of the cow stable stir up large quantities of dust and fill the air with microörganisms. It is astonishing to see how many bacteria can adhere to a small piece of hay or may be found in a gram of any of our common dairy feeds. When these materials are fed dry just previous to milking time, the atmosphere of the stable will be filled with organisms which may settle into the milk while it is exposed during the process of milking. The effect of this source of contamination may be seen by the following experiments:

BACTERIAL CONTENT OF MILK AS AFFECTED BY FEEDING DRY HAY AND GRAIN

Experiment	Date	Nature of sample	Number bacteria per c.c.
No. 1	May 4	Before feeding	350
		After feeding	1,450
No. 2	May 17	Before feeding	2,900
		After feeding	4,400
No. 3	May 18	Before feeding	4,100
		After feeding	7,200

DAIRY UTENSILS.—All utensils which are to be used in connection with milk should be so constructed that there are no cracks or crevices in which the milk can accumulate and from which it is not easily washed. A milk pail with an open seam may be the cause of serious trouble in the dairy. The dairy utensils should be simple in construction and so made that they can be thoroughly cleansed with ease

and made of such material that they can be thoroughly sterilize either with water which is actually boiling or in steam.

THE MILKER.—No food material requires greater care and cleanl ness on the part of those handling it than does milk. All persons havir to do with the handling of this delicate food product should constant keep in mind that clean hands and clothing and extreme cleanliness every operation is very necessary if milk of good quality is to be o tained.

GROUPS OR TYPES OF MICROÖRGANISMS FOUND IN MILK AND THEIR SOURCES

In studying the types of bacteria found in milk, it is convenie to arrange them in groups based upon their action on the milk a their effect upon persons consuming it. There are certain types organisms which are very troublesome to the milk dealer but which a not injurious to the consumer. Other species which may be of little no significance from their action on the milk are of greatest significan from the standpoint of the consumer since most of the disease organis which may be carried by milk have no appreciable action upon it. S other forms are of but little importance to either the dealer or the co sumer and others are troublesome to both.

GENERAL SIGNIFICANCE OF ACID-FORMING BACTERIA.—Of all t bacteria that find their way into milk, those that are able to ferment t milk sugar, producing from it different kinds and amounts of acids, fi more favorable conditions for growth at ordinary temperatures, 15° 45°, than do those belonging to other groups. Because of their grea rapidity of growth and because of the inhibiting effect of their by-pr ucts upon the other groups of bacteria, the acid types tend to predo inate in milk and the specific change which they produce, the souri is of such common occurrence that it is often looked upon as somethi inherent in milk.

GROUPS OF ACID-FORMING BACTERIA.*—The acid-forming bacte that are constantly present in milk represent many kinds which differ morphology, in cultural characteristics, and in their products of ferm tation. They may be divided into four groups that vary greatly as as their importance in the handling of milk is concerned. If milk is p duced under clean conditions and is kept at temperatures ranging fr

* Prepared by E. G. Hastings.

r to 35°, the acid fermentation will be almost wholly due to a group of bcteria closely allied to one of the pathogenic forms, *Strept. pyogenes* (osenbach). To representatives of this group, which is of the greate importance in all phases of dairying, have been given various names b different investigators. The most important organism of this group is ne to which the name *Bact. lactis acidi* is applied. The group undoubtedy includes a large number of organisms, all of which produce, however, a similar change in milk.

Second in importance is a group of organisms, of which the best kown representatives are *B. coli communis* and *Bact. lactis aerogenes*. A large number of organisms of this group have been described and named. The most important characteristics of the representatives mentioned will, however, suffice to characterize the group. A third group is represented by *Bact. bulgaricum* and the rod-shaped organisms that have been studied especially by de Freudenreich. A fourth group includes many acid-forming cocci, some of which exhibit proteolytic properties while others do not. Organisms of the third and fourth groups exert little or no effect in the normal acid fermentation of milk, although they are constantly present in varying numbers, as can be demonstrated by appropriate means, and are of importance in certain phases of dairy manufacturing.

In any sample of milk the relative number of bacteria belonging to each of the first two groups is dependent upon the conditions surrounding production, especially with reference to cleanliness. The bacteria belonging to the first group come largely from the milk utensils and are also found in the dust of the barn and on the coat of the animal. The source of the second group is largely the fecal matter that gains entrance to the milk, although they are also found in the upper layers of the soil and on grain. They are introduced into the milk with the dirt. The cleaner the conditions of production, the smaller will be the number of these two groups of organisms found in fresh milk.

The manufacture of the leading type of butter and of all kinds of cheese is dependent on the action of microörganisms, hence dairy manufacturing should be classed as a true fermentation industry. In all such industries one of the factors determining the quality of the product is the type of microörganism employed to produce the desired fermentation, and the importance of insuring the presence of desirable organisms, and the exclusion of harmful kinds is well recognized.

The most important properties of organisms employed in the fermentation industries are the physiological rather than the cultural or morphological, since the quality of the product is dependent on the by-products of the fermentation. Hence in characterizing the groups of acid-forming bacteria, the biochemistry of each group will be emphasized rather than the cultural and morphological characteristics of the members of the group.

*Characteristics of the Bact. Lactis Acidi Group.**—The organisms of this group are widely distributed in nature, as is shown by the constancy with which milk undergoes the characteristic fermentation produced by the members of the group.

The cells are oval in form, about 0.6μ to 1μ in length, and 0.5μ in diameter. The shorter cells appear nearly spherical, which, together with the fact that chains of cells often occur, has led some to classify them among the cocci and Kruse has applied the name *Strept. lacticus* to a member of the group. In milk the cells are usually in twos, the outer ends of the two cells being pointed. None of the group is motile; spores are not formed and capsules are often noted. The members of the group are Gram-positive.

The optimum temperature for growth lies between 30° and 35°, the minimum growth temperature ranging from 10° to 12°, while the maximum is 12°. They are to be classed as facultative aerobes. The growth on all culture media is marked by its meagerness; in the absence of a fermentable carbohydrate, no growth usually occurs; peptone favors the growth even in milk. In the case of freshly isolated cultures, the growth is almost invisible, on slopes of sugar agar appearing as small discrete colonies. On sugar agar plates the colonies are small, often surrounded by a hazy zone, and always occur below the surface of the medium. In lactose-agar stab cultures growth occurs along the entire line of inoculation, but there is no surface growth. No liquefaction of gelatin occurs. In bouillon the medium is uniformly turbid or it remains clear with a slight sediment. On potato, growth is slight or is absent. Milk is usually curdled within twenty-four hours at the optimum temperature by members of the group, although some fail to curdle the milk, since the maximum amount of acid produced is not sufficient to cause this phenomenon. Still others cause curdling in the presence of small amounts of acids, in which case a rennet-like enzyme may

* Prepared by E. G. Hastings.

b present. No gas is produced in the fermentation of lactose, hence t curd formed in milk is perfectly homogeneous; it shows but little t dency to shrink and to express whey. In litmus milk the color is d charged from the entire mass of medium before curdling occurs, due t the reduction of the litmus to the colorless leuco-compound. Through t action of the oxygen of the air the litmus is slowly reoxidized and t pink layer, which immediately after curdling is but a few millimeters i depth, is slowly extended until the entire mass of curd has a uniform p k color. Saccharose, dextrose, maltose, and mannit are fermented.

The maximum amount of acid produced by organisms that are most t ical of the group is determined by the composition of the medium. I is often said that the organisms causing the normal souring of milk r present a group than can grow in a strongly acid medium. This is t e as far as acid salts are concerned, but free acid totally inhibits g wth. In a culture medium, which contains no substance that can c nbine with the acid formed and thus remove it from the sphere of a ion, no growth, or but very slight growth occurs. In sugar bouillon a l in milk, the amount of acid formed is determined by the amount of s stances in these liquids that can combine with the acid. In milk s h compounds are the casein and some of the ash constituents, e ecially the phosphates. In normal milk, the maximum acidity a ained ranges from 0.9 to 1.25 per cent calculated as lactic acid. If t content of neutralizing compounds per unit volume is varied by c centration, dilution, or by the addition of such substances as cal- c m phosphate, the maximum amount of acid produced by typical c tures will be changed. In sugar bouillon the maximum acidity p duced rarely exceeds 0.25 per cent.

The fermentation of lactose is usually expressed as follows:

$$C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3.$$

T s 342 parts of lactose should yield 360 parts of lactic acid. The t oretical yield of lactic acid is never obtained, for the action of the o anism on the carbohydrate is much more complex than is represented b the equation given. In the following table are given data obtained b a number of investigators.

These data signify that other compounds than lactic acid are f med in the fermentation of lactose by these acid-forming bacteria. A tic acid ($CH_3.COOH$); formic acid (H.COOH); propionic acid

Sugar content of milk, per cent.	Sugar fermented, per cent.	Lactic acid calculated, per cent.	Lactic acid found, per cent. of theoretical
4.54	0.60	0.632	89.56
4.96	0.56	0.590	98.13
4.94	0.65	0.684	97.89

($C_2H_5.COOH$); traces of alcohols, aldehydes and esters have bee found. The lactic acid formed is the dextro modification. It is be lieved that the fermentation is due to an enzyme, lactacidase, one of th intracellular enzymes that can be demonstrated only with difficulty.

Milk fermented by members of this group has a mild acid taste, a agreeable odor, and the curd can be so finely divided by agitation as t produce almost as perfect an emulsion as in raw milk. The organism are to be classed as desirable from the standpoint of the dairy manu facturer, and the fermentation produced by them may be called a tru *lactic* fermentation.

*Characteristics of the B. Coli-aerogenes Group.**—This group includ a considerable variety of organisms, which differ in morphology, in cu tural characteristics and undoubtedly in the character and amounts their by-products. They are more distinctly bacilli than the membe of the preceding group; are motile or non-motile; none produces spor and they are usually negative to Gram's stain. The optimum grow temperature, 35° to 40°, is somewhat higher than for the precedi group, the vegetation range being 15° to 45°. They are to be classed facultative anaerobes.

The conditions for development are less narrow than for the *Ba lactis acidi* group, growth occurring on all the ordinary culture med and in the absence of carbohydrates. Indol and hydrogen sulphi are often formed and nitrates are reduced. The growth is usually pr fuse, the colonies large and surface growth occurring in stab cultur Gelatin is not usually liquefied.

Lactose, dextrose and saccharose are fermented, with the producti of varying amounts of gas in which have been found carbon dioxi hydrogen, methane, and free nitrogen. The maximum amount of a produced in any culture medium is quite similar to that formed by t members of the previous group. The relative proportions between t

* Prepared by E. G. Hastings.

non-volatile and volatile acids are far different, lactic acid comprising less than 30 per cent of the total acid formed, while volatile acids, such as acetic and formic, make up the remainder. Traces of succinic acid ($CH_4(COOH_2)$) and alcohol have also been found. The lactic acid is of the lævo-form.

Milk is usually curdled, although some members of the group do not produce enough acid to cause curdling. Depending on the amount of acid formed, the curd may be almost perfectly homogeneous or it may be very spongy. In all cases the curd shrinks to a greater or less extent and thus becomes so firm that it is difficult or impossible to emulsify it again. The odor of the fermented milk is offensive and the taste disagreeable and sharp. The organisms of this group are to be classed as undesirable and the fermentation produced by them cannot correctly be called a lactic fermentation.

Representatives of these two great groups of acid-forming bacteria are to be found in every sample of market milk in varying proportions. Both find in milk favorable conditions for growth, and the normal souring is produced conjointly by them, each producing its own specific products, the relative amounts of which are largely dependent on the number of each group that is originally introduced into the milk. The value of milk for butter and cheese is determined by the relative amounts of the products of the desirable and the undesirable acid-forming bacteria.

The difference in taste and odor between milk fermented by pure cultures of *Bact. lactis acidi*, and that which has soured spontaneously, emphasizes the difference in the products of the fermentations produced by the two groups of acid-forming bacteria.

*Characteristics of the Bact. Bulgaricum Group.**—The organisms of this group are to be classed as true lactic bacteria, since they produce almost exclusively lactic acid from the sugar fermented and only small quantities of other acids as formic, acetic, and propionic. They vary widely in form and size; but are usually large rods, 2μ to 3μ long and 0.4 to 1μ wide. There is a tendency to form long threads. They are Gram-positive and when stained with methylene blue often show distinct granules in the cells; with Neisser's stain the appearance of some cultures is similar to that of the diphtheria bacterium. They are non-motile and do not form spores; capsules are seldom noted. The

* Prepared by E. G. Hastings.

optimum growth temperature is from 40° to 50° and the minimum asserted to be 25°, although for many members of the group it must much lower.

The growth on all ordinary culture media is meager or is abser the colonies are often microscopic in size and show radiating threa Free acids do not inhibit development and the term *acidophilous* h been applied to the group. They grow slowly in milk, even at t optimum temperature, and curdling may not occur for several da the curd is homogeneous and in litmus milk reduction occurs. T maximum amount of acid varies from 1.25 to 4.0 per cent. So members of the group produce dextro-, others lævo-acid, and racen acid is formed in some cases. The curd may be easily broken by agi tion, and through the solvent action of the acid is partially dissolv The organisms do not liquefy gelatin, but the casein of milk is partia changed into soluble decomposition products, as was first shown by Freudenreich, and later confirmed by Hastings.

It has been supposed by many that this group was confined and characteristic of certain of the fermented milks, especially th of eastern Europe and western Asia, such as Yogurt and Matzo Recent work has shown that this group is widely distributed in natu Representatives of this group are found constantly in milk and ot dairy products. Their presence in milk can be demonstrated placing a sample of milk in a corked bottle, and incubating at 37°. T acidity of the milk increases rapidly at first, due to the growth of members of the two previous groups. These ordinary acid-form organisms are soon inhibited by the appearance of free acid, but acidity of the milk nevertheless continues to increase slowly, a with this continued increase a change in flora is noted, the sh plump bacilli ceasing to predominate and long slender rods constan increasing in numbers. The source of this group is undoubte the alimentary tract of the animal.

*Characteristics of the Coccus Group.**—This group is well represen by the bacteria which form the characteristic flora of the udder. T vary greatly in size and in other properties. They retain Gra stain; many are chromogenic, the color ranging from a white t deep orange. They grow slowly on all ordinary culture media, the growth is not necessarily meager. Generally they are aero

* Prepared by E. G. Hastings.

though many grow under anaerobic conditions. Gelatin may be quefied or not. Milk may or may not be curdled, the curd often sembling that formed by rennet-like enzymes. They produce no ctic acid, but only acetic, propionic, butyric and caproic acids, d hence cannot be classed as lactic bacteria.

Bacteria Having No Appreciable Effect on Milk.—This oup is made up of many different forms. They produce no changes hich can be detected either by the eye or the taste. They do not velop very rapidly in milk, and some species gradually disappear hile others increase in numbers. Many of the organisms in this oup are chromogenic, orange and lemon yellows being among the ore common forms. They are mostly cocci and do not liquefy gelatin. om the standpoint of the commercial milkman these organisms e of little significance and this is probably also true from the stand-int of the consumer.

The Casein-digesting or Peptonizing Bacteria.—These organ-ms digest the casein either with or without coagulation. Many of em coagulate the casein with an alkaline reaction. They liquefy latin. Most of the organisms of this group are rods of various shapes d sizes, some of them being the largest rods found in milk. Some are otile and some non-motile. Some representatives of this group oduce little or no odor, but many of the species develop very strong trefactive odors. Barny or cowy odors or other off-flavors sometimes und in milk and dairy products may be caused by the action of this pe of bacteria. They are associated with filth and their presence milk indicates insanitary conditions of production or handling.

Pathogenic Organisms.—This group includes all those species hich may gain access to milk, which are capable of causing specific seases in human beings. They are of the greatest importance to the nsumer. They do not appreciably affect the physical or chemical operties of the milk, or produce any changes in its appearance, vor, or keeping quality which would indicate their presence. me of them do not even develop in milk, as is the case with the *Bact. berculosis*. Others, as the diphtheria bacteria and typhoid fever cilli, may grow in milk with great rapidity. This group also con-ins certain species which produce diarrhœal disorders, especially infants and young children. Some of them are probably organisms hich are also included in the peptonizing group. The specific

pathogenic organisms, possibly with the exception of *Bact. tuberculosis*, get into milk, either directly or indirectly, from human patients suffering with the particular disease.

Factors Influencing the Development of Microörganisms in Milk

—The number of microörganisms found in fresh milk shows its bacterial condition at that time, but it gives little idea of the organisms which may be found in the same milk at later periods. There are many factors to be considered if we wish to study the development of the various types which get into ordinary milk. These factors may be considered briefly under the following heads:

Initial Contamination.—Fresh milk varies widely in the number of organisms which it contains as a result of the conditions under which it has been produced. There are differences not only in the numbers of organisms but also in the species which may be found in different samples of fresh milk. Both of these factors are important in the later changes which may take place. The effect of numerical initial contamination may be seen in the following tables where

Effect of Initial Contamination on Development of Bacteria and Keeping Quality of Milk

Milk Having Moderately High Initial Contamination

Bacteria per c.c. in fresh milk	Bacteria 12 hours	Bacteria 36 hours	Hours to curdling
187,000	432,000	633,500,000	45

Milk Having Moderate Initial Contamination

Bacteria per c.c. in fresh milk	Bacteria 12 hours	Bacteria 36 hours	Hours to curdling
3,000	14,000	149,650,000	99

Milk Having Small Initial Contamination

Bacteria per c.c. in fresh milk	Bacteria 12 hours	Bacteria 36 hours	Hours to curdling
325	1,712	10,125,000	121

ilk starting out with different numbers of organisms was kept under imilar conditions until coagulation. Plate cultures made from these hree samples show the relative development of the number of rganisms.

These samples were all kept at a constant temperature of 21° and he difference in the numbers of bacteria and the curdling time can herefore be fairly attributed to the difference in the initial contamina- ion of the three samples. All three of the samples showed a normal evelopment of the lactic organisms, which constituted over 99 per ent of the total organisms present at the time of curdling. While his may be considered as showing the normal effect of the original ontamination upon the milk, it is well to bear in mind the fact that here are many apparent exceptions due to some particular type of rganism predominating and interfering with the normal development f the lactic types.

STRAINING.—The straining of milk is one of the most common perations in connection with its handling and is considered by most airymen as one of the most essential from the standpoint of the qual- y of the milk. If milk is strained through cheese cloth or wire auze much of the insoluble dirt can be removed. This has led to the eneral belief that straining improves the sanitary and keeping quali- es of the milk.

The effect of straining on removal of insoluble dirt is shown by he following results of the tests:

DIRT REMOVED BY PASSING MILK THROUGH TWO THICKNESSES OF FINE CLOTH
(Weight of insoluble dirt given in milligrams per liter of milk)

Experiment	Before straining	After straining	Per cent removed
o. 1	8.95	4.70	47.5
o. 2	5.55	4.95	10.8
o. 3	5.15	2.95	42.7
o. 4	2.45	0.20	91.8
o. 5	5.05	3.10	38.6

It may be noticed that even after straining the milk contained ppreciable quantities of insoluble dirt which had passed through he strainer cloth. The difference in per cent of dirt removed in

different samples is due to the nature of the dirt itself. The coarser the dirt the greater the proportion that will be removed by straining.

It is not true, however, that the keeping quality is necessarily improved by the simple process of straining. It depends largely upon the condition of the milk and the nature of the strainer. Not infrequently passing milk through a strainer not only fails to improve its keeping quality but actually injures it. This has been shown by a number of investigators. The effect of straining upon the germ content may be seen in the following figures where the milk was passed through a strainer composed of three thicknesses of fine cheese cloth supported by wire gauze.

EFFECT OF STRAINING UPON BACTERIAL CONTENT OF MILK

Experiment	Before straining, bacteria per c.c.	After straining, bacteria per c.c.
No. 1	3,600	3,600
No. 2	7,400	6,900
No. 3	12,800	10,500
No. 4	8,800	11,375
No. 5	8,800	2,700

The effect of straining upon the keeping quality is shown in the following experiments where the milk was strained through the same form of strainer mentioned above and the samples kept at constant temperature of 21° until coagulation.

EFFECT OF STRAINING UPON KEEPING QUALITY OF MILK

	Not strained, hours to coagulation	Strained, hours to coagulation
Experiment No. 1	42	42
Experiment No. 2	57	55
Experiment No. 3	35	35
Experiment No. 4	89	54
Experiment No. 5	50	50

It will be seen that in no case was the keeping quality of these samples increased by the straining process while in some cases it was materially injured.

Cotton filters are more efficient than cheese cloth and in some ases the keeping quality of the milk may be improved by this process.

Aeration.—This is the process of exposing the milk to the atmos- here by allowing it to run over the surface of the aerator in a very hin film. If milk has been produced under such conditions that it as absorbed foreign odors, this process may be of value in getting d of the absorbed odors, but from the bacterial standpoint the process f aerating is not desirable, since it gives one more opportunity for he milk to become contaminated with organisms from the atmos- here and from the aerator itself. It is possible to aerate milk under uch conditions that the germ content will not be increased, but if eration takes place in the cow stable or other place where the atmos- here contains dust the number of organisms will be greater after eration than before, the amount of increase being proportional o the sanitary conditions under which the aeration is done. It is ven possible that the milk may absorb foreign odors during the proc- ss of aeration and be of poorer quality than it was before. It is thought y many that the process of aeration is necessary in order to get rid f the so-called animal odors commonly found in milk. These odors re, however, not normal to the milk but are absorbed from the foul ir in the stables or other sources. This is shown by the fact that some f the very finest quality of certified milk is bottled while still con- aining the animal heat with the least possible exposure to the air, ghtly sealed at once and plunged into ice water. Such milk contains o suggestion of animal odor. Aeration may be of value in removing ndesirable odors from milk which is not produced under good anitary conditions, if done in an atmosphere free from all dust and dors, but it is not necessary for milk of good quality. The common elief that aeration is valuable is probably due to the fact that most erators are coolers as well, and the beneficial results are due to the ooling and not the aeration.

Centrifugal Separation.—It is a common practice in some dairies o pass the milk through a centrifugal separator or clarifier to emove any dirt which it may contain. This operation is effective or the removal of much of the insoluble dirt which may be in the milk, ut it is of undetermined value as yet from the standpoint of the acterial content and the keeping quality of the milk. In spite of the act that the separator slime is very rich in bacteria, the milk and

cream as they come from the machine will normally show larger bacterial counts in agar and gelatin plates than will the milk before treatment, due of course to the breaking up of colonies. The usual effect upon the germ content of passing milk through a separator or clarifier may be seen in the following tables:

INFLUENCE OF PASSING MILK THROUGH A CENTRIFUGAL SEPARATOR UPON THE GERM CONTENT OF THE SKIM MILK AND CREAM

	Bacteria in whole milk	Bacteria in skim milk	Bacteria in cream
Sample No. 1.....	39,000	69,000	75,000
Sample No. 2.....	44,000	76,000	790,000
Sample No. 3.....	56,000	75,000	820,000
Sample No. 4.....	200,000	336,000	330,000

EFFECT OF A CENTRIFUGAL CLARIFIER UPON THE GERM CONTENT OF MILK

Sample number	Bacteria before clarifying	Bacteria after clarifying	Numerical increase	Percentage increase
1	6,000	9,000	3,000	50
2	15,000	22,000	7,000	46
3	60,000	156,000	96,000	160
4	133,000	197,000	64,000	48
5	370,000	643,000	273,000	73

These increased counts do not mean that there is an actual increase in individual bacteria in these samples due to the action of the separator or clarifier. What it does mean is that the small clusters or groups of organisms, as they exist in the whole milk are thrown apart by the centrifugal force and therefore develop individual colonies in the plate cultures.

TEMPERATURE.—The temperature at which milk is kept is one of the most important factors determining the development of its microbial content. Every one at all familiar with milk knows that it spoils very quickly if allowed to stand at warm temperatures. If, however, the milk is held at temperatures of 10° or lower, the keeping quality of the milk is greatly increased. Most of the ordinary species of organisms which gain entrance to milk do not grow rapidly at temperatures of 10° or lower. There are, however, certain species

hich will grow with considerable rapidity at temperatures below 10°, pecially some of the spore-bearing non-acid forms. If the tempera- re of the milk is allowed to rise above 10°, the growth of the common ecies increases rapidly. The influence of temperature upon the velopment of bacteria may be seen in the following experiment here a given lot of milk was thoroughly mixed and divided into ven portions, which were then held at the temperatures indicated r twelve hours, at the end of which time they were plated for the tal germ content.

FECT OF DIFFERENT TEMPERATURES UPON THE DEVELOPMENT OF BACTERIA IN MILK

Temperature maintained for 12 hours		Bacteria per c.c. at end of 12 hours	Hours to curdling at 21°
C.	F.		
4.5°	40°	4,000	75
7°	45°	9,000	75
10°	50°	18,000	72
12.5°	55°	38,000	49
15.5°	60°	453,000	43
21°	70°	8,800,000	32
26.5°	80°	55,300,000	28

The fresh milk showed a count of 5,000 per c.c. and curdled in ty-two hours at a temperature of 21°. The curdling time of these mples was determined by placing them at a constant temperature 21° at the close of the twelve-hour period and holding them at this mperature until coagulation took place. The difference in time of rdling therefore is due to the maintenance of the special tempera- re for twelve hours only and not for the entire period up to the time curdling.

PASTEURIZATION.—The term pasteurization is used to designate e process of heating milk to a temperature sufficient to destroy portion of the bacteria and then cooling it to a temperature which ill prevent the rapid development of the organisms that are left. he temperatures commonly used for this purpose vary from 60° to °. The length of time the milk is exposed to the high temperature ay also vary from a few seconds to thirty minutes, depending upon e method employed. The two chief purposes for the pasteuriza-

tion of milk are to increase its keeping quality and to destroy any pathogenic organisms which the milk may contain. The purpose for which the pasteurization is done will determine the method used. In commercial pasteurization, where the chief purpose is to destroy the lactic organisms and thus improve the keeping quality of the milk, the method used is that known as the "flash" or instantaneous method, where the milk is subjected to a high temperature for a few seconds only and then cooled. In this method of pasteurization varying degrees of efficiency are obtained, depending upon a number of factors, chiefly the bacterial condition of the milk to be pasteurized, the degree of heat and the length of the exposure and the temperature to which the milk is cooled. By this method, it is possible to destroy a large percentage of the organisms in the raw milk, and materially increase its keeping quality, but the temperature and time to which any particle of milk is exposed cannot be accurately controlled, and this method cannot be depended upon to kill all of the disease-producing organisms which may be in the milk. This method has been largely abandoned for the pasteurization of market milk.

Where the chief purpose of pasteurization is to render the milk free from disease-producing organisms, the so-called "holding" method is employed. This consists in raising the temperature of the milk to about 60° to 63° and holding it at this temperature for a period of twenty to thirty minutes. If this method is properly done, most of the organisms except certain spore forms should be killed and the milk at the end of the pasteurizing process contain only a small percentage of its original germ content.

Formerly it was believed that heating milk to a high temperature killed all the lactic acid organisms, and favored the subsequent growth of other more undesirable species, but more recent studies on the bacteria flora of milk, pasteurized by the "holding" method, have shown that some strains of the lactic acid bacteria can survive the relatively lower temperatures used in this method, and that the later development of the different groups of bacteria is similar to that in raw milk of equal bacterial grade.

Pasteurization at the temperatures used in the holding process does not seem to cause any injurious chemical changes in the milk constituents, or affect its digestibility.

Proper pasteurization gives a valuable means of rendering the milk

supply for our cities reasonably free from pathogenic microörganisms, but, in order to insure this safety, the work must be carefully done, and all later contamination avoided. Preferably, the work should be done under expert, municipal supervision. Undoubtedly the ideal method is pasteurization in the sealed bottle which is to be delivered to the consumer, since this method reduces to the minimum the danger of subsequent contamination.

Pasteurization must not be regarded as a substitute for care and cleanliness or a means of renovating old or dirty milk otherwise unfit for use, but rather as an additional means of protecting the consumer against disease-producing microörganisms in the milk supply.

The Use of Chemicals.—The addition of certain chemicals to milk will retard the growth of bacteria. The chemicals most commonly used for this purpose are calcium hypochlorite, borax and formalin. While the keeping quality of milk may be materially increased by the use of such chemicals, their use has been opposed by health authorities and is contrary to the Pure Food Laws. If milk is handled with any degree of care, there should be no need for the use of chemical preservatives. They are simply a means of counteracting the unsanitary conditions of the production and handling. The same results can be obtained by cleanliness in the production of the milk and the use of low temperatures for preventing the contamination and subsequent growth of the bacteria in the milk. The developments in the production of clean milk of the past few years have illustrated very clearly that the use of chemical preservatives is not necessary.

Normal Development of Microörganisms in Milk

The flora of any particular sample of fresh milk is determined by the conditions under which it is produced. In stables where extreme cleanliness is practised the flora may be practically limited to those species which occur in the udder of the cows, but under ordinary conditions there will be in addition to the normal udder types such others as may occur on the cow's body and in the dust and atmosphere of the stables. Market milk, therefore, when first obtained from the cow ordinarily contains a mixed flora, the different types present depending upon the sanitary conditions under which the milk is produced.

The future development of this initial flora is largely dependent

upon the temperature at which the milk is kept. If the milk is held a temperatures between 10° and 21° there will result what may be con sidered as the normal development of milk fermentations. Thes changes may be divided for convenience into four periods or stages.

FIRST STAGE. GERMICIDAL PERIOD.—It has been shown by a num ber of investigators that instead of an increase in the numbers of bacter in fresh milk there is normally a decrease in the number during th first few hours after its production. The rapidity of this decrease an the length of time over which it extends seem to be determined large by the temperature at which the milk is kept. The higher the temper ture the more rapidly the number of organisms decreases and the mo quickly the end of the germicidal period is reached. If the temper tures are kept fairly low the rate of decrease is much slower but the d cline will extend over a considerably longer period. This is shown l the following examples given by Hunziker.

TABLE SHOWING THE GERMICIDAL ACTION IN COW'S MILK

Name of cow	Milk, warm and fresh	Temp.* of milk	After 3 hours	After 6 hours	After 9 hours	After 12 hours	After 15 hours	After 24 hours	After 32 hours	Aft 4 hou
May.....	1,212	40°	1,080	1,220	1,040	1,020	1,120	1,360	1,040	
		55°	1,260	1,400	1,500	1,462	1,360	1,080	3,500	17,
		70°	1,000	1,340	1,860	3,460	3,460	64,000	800,000	
Ida.....	5,120	40°	4,400	4,260	3,620	3,700	3,900	4,000	3,900	3,
		55°	3,900	3,460	2,980	2,800	2,920	3,260	3,220	3,
		70°	3,560	2,120	1,880	1,880	1,240	4,960	58,400	
Julia.....	1,345	40°	1,170	1,070	1,120	870	1,120	990	1,060	1,
		55°	1,080	990	980	1,400	1,080	1,080	3,110	68,
		70°	1,000	1,000	1,200	5,600	17,720	1,600,000		

The exact reason for this decline is at present not well understo Some investigators believe that milk possesses a certain germicidal tion or property which results in the destruction of a portion of organisms found in the milk at the outset.

The work of other investigators seems to show that the so-cal germicidal action is felt by certain species and not by others as is ir cated by the following sample.

* Fahrenheit.

Age of milk	Total bacteria	Acid bacteria	Per cent. acid bacteria	Liquefying bacteria
Fresh.....	12,550	1,250	10	200
3 hours.....	12,250	2,000	16	200
6 hours.....	19,650	2,250	23	800
9 hours.....	56,900	20,250	36	550
12 hours.....	114,250	68,400	60	1,900

This would seem to indicate that the decrease in number is due not so much to a definite germicidal property possessed by the milk as to the gradual dying out of certain species which for some reason do not find the milk a suitable environment for development, while other types, finding the milk suitable to their needs, develop uniformly from the start.

Rosenau and McCoy found that the germicidal properties of milk were destroyed by boiling or by heating it above 80° and that lower temperatures destroyed it for certain organisms. These workers also found that there was marked agglutination of the organisms in raw milk and conclude that this accounts for the decreased number of colonies developing in plate cultures and that the germicidal action is therefore more apparent than real.

Second Stage. Period from End of Germicidal Action to Time of Curdling.—The period following immediately after the germicidal action is characterized by the rapid development of the lactic organisms. Under normal conditions this group develops much more rapidly than any other type. Not only do they increase rapidly in actual numbers but their percentage also rises rapidly. There may be continual increase in numbers in the other species, but their growth is much less rapid than that of the *Bact. lactis acidi* type. As this period advances certain of the miscellaneous types may cease to grow entirely. During this time the gas-producing acid organisms of the *B. coli* and *Bact. lactis aerogenes* type may develop more or less rapidly, but if the milk is held at temperatures not much above 20°, the *Bact. lactis acidi* type will develop much more rapidly, so that by the time the milk becomes sour and curdles, this type will constitute 99 per cent approximately of the total number in the milk. From the standpoint of the milk consumer milk ceases to be of value when the end of this period is

reached, but there are further developments which are of importance certain lines of dairy manufactures, notably cheese making.

THIRD STAGE. PERIOD FROM TIME OF CURDLING UNTIL ACIDI IS NEUTRALIZED.—At the time milk curdles it contains enormous nu bers of the lactic bacteria. The number usually runs into the millic and may be even higher than one thousand million per c.c. By t time the coagulation takes place the acidity of the milk is so high th the growth of the lactic organisms is checked and from this time their number decreases with more or less rapidity.

During the period following the curdling certain other types organisms which have existed in the milk during the earlier stages n begin to grow. The organisms especially important in this stage *Oidium lactis*, certain species of molds, and yeasts. These organis are able to grow in a highly acid medium, and as a result of th development the acid is decreased until the milk finally presents neutral or alkaline condition resulting from the decomposition of proteins in the milk.

FOURTH STAGE. FINAL DECOMPOSITION CHANGES.—The reduct of the acidity affords favorable conditions for the growth of certain ty of organisms which have remained in the milk during the earlier sta but have been practically dormant. In this fourth stage the conditi are suitable for the growth of the liquefying and peptonizing bacte and they now grow rapidly, causing the decomposition of the case. The changes resulting from this type of organisms are of special sign cance in cheese making and are discussed more fully in another chap

ABNORMAL FERMENTATIONS IN MILK

GASSY FERMENTATION.—It frequently happens that instead of normally rapid development of the *Bact. lactis acidi* type of organis in the milk, other acid producers develop rapidly, with the product of more or less gas. The organisms most prominent in this type of mentation are the *B. coli communis* and the *Bact. lactis aerogenes* ty This group of organisms contains a number of varieties, some of wh produce little or no gas while others develop large amounts. Th action in milk is usually accompanied by disagreeable odors and flav They grow readily in the presence of air and therefore develop abund colonies on the surface of plate cultures. This distinguishes the m

bs of this group quite clearly from those of the true lactic group which gw chiefly below the surface of the medium. The members of this gup do not form spores, but certain varieties are quite resistant to ht and will oft times survive pasteurizing temperatures which comptely destroy the *Bact. lactis acidi* group. They grow most rapidly a high temperatures, between 20° and 37°.

Sweet Curdling Fermentation.—This phenomenon is caused by a variety of organisms which cause the milk to coagulate without the pduction of acid. The coagulation is brought about by a rennet-like eyme produced by this type of bacteria. The resulting milk is eher neutral or alkaline in reaction. Usually the coagulation of the nk is followed by the digestion of the casein as a result of another eyme which is also produced by these bacteria. The coagulation cised by these organisms is slower than in the case of the acid fmers and the curd is usually soft and mushy as compared with the cd formed in the normal acid fermentation. The members of this gup get into the milk from and along with dust ad dirt associated with unsanitary conditions. Sme of the species produce spores and are not lled by the ordinary methods of pasteurization. his fact accounts for the occurrence of sweet cdling of pasteurized milk. This group of orgnisms is unable to develop rapidly in the presce of the lactic bacteria and for this reason we not commonly get the sweet curdling of raw lk. The presence of these organisms is evince of insanitary conditions. Frequently they velop very disagreeable flavors in the milk.

Fig. 131.—Ropy cream lifted with a fork. (*After Ward.*)

Ropy or Slimy Fermentation.—One of the st common milk infections causing trouble to e milk dealer is that which causes a ropy or my fermentation of milk. This is sometimes spoken of as stringy lk (Fig. 131). Several species of organisms are capable of procing this condition. These organisms grow most freely in the esence of an abundant supply of oxygen and for this reason the cream ually becomes slimy before any changes are apparent in the undering layers of milk. *B. lactis viscosus* is perhaps the most common ecies in this group. The slimy condition in the milk is supposed

to be the result of a very viscid capsule surrounding these organisi (Fig. 132). Representatives of this group are quite resistant to he and frequently pass uninjured through the methods of cleansing a scalding used under ordinary dairy conditions. Because of th dairy utensils once infected become a constant source of infectic

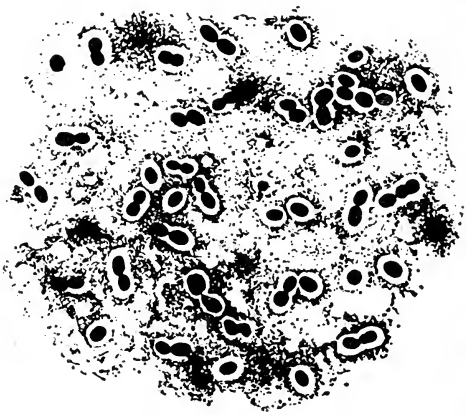


FIG. 132.—*Bacillus lactis viscosus* from a milk culture. (*After Ward.*)

This trouble can be effectively stopped by a thorough scalding of utensils coming in contact with the milk.

BITTER FERMENTATION.—Bitter flavors in milk may be the res of bacterial changes after the milk has been drawn, or due to certa feeds which the cows have consumed. If the cows are allow to eat certain kinds of vegetation, such as "rag weed" and certa other plants, they may impart a bitter taste to the milk, in which ca the abnormal flavor will be apparent when the milk is fresh and usua becomes less pronounced as the milk becomes older, because of t volatile nature of the substances causing the bitterness. Most of t cases of bitter milk and cream, however, are due to the growth certain types of bacteria in which case the bitterness increases in tensity with the age of the milk. Some of the species capable producing bitter milk grow at quite low temperatures, which accou for the fact that the most trouble with bitter flavors is found in m and cream which has been held at low temperatures for some tir

Alcoholic Fermentation.—The bacteria as a group are not able to act on the milk sugar and produce alcohol, but it sometimes happens that yeasts get into the milk in sufficient numbers to ferment the milk sugar, producing appreciable amounts of alcohol. To the milk handler this trouble is not usually serious but the action of the yeasts is frequently of considerable importance in the cheese industry.

Other Fermentations.—It frequently happens that a considerable variety of disagreeable flavors and odors develop in milk. These may be due to the direct absorption of odors from the foul stable atmosphere or strong-smelling feeds, such as silage; or they may be, and no doubt frequently are, the result of the growth of certain types of bacteria which have entered the milk from dirty surroundings. The growth of some of these organisms is frequently the cause of the so-called cowy and stable odors and flavors.

Commercial Significance of Microörganisms in Milk

Relation of Dirt Contamination to Germ Content.—To the commercial milkman bacteria are of importance only as they influence the length of time the milk will keep in a salable condition. The consumers do not want milk that is sour or has unpleasant flavors and odors. In order to sell his milk, therefore, the milkman must avoid the presence of these undesirable conditions, and in proportion as he recognizes the relation between germ life and the quality of his product, will he pay attention to the presence and development of microörganisms in his milk. In like manner, the presence or absence of dirt contamination is important from the commercial standpoint since it bears a relation to the bacterial count, and, therefore, affects the keeping properties of the milk. Under normal conditions there is a fairly direct relation between the amount of visible or soluble dirt and the number of bacteria found in any given lot of fresh milk. This relation may be shown by the following samples taken from four different milk producers:

Producer	Number of samples	Average mg. dry dirt per liter	Average number bacteria per c.c.	Average hours to time of curdling
A.....	5	51.5	115,000	175
B.....	16	58.8	273,600	78
C.....	21	70.0	428,600	75
D.....	17	71.9	949,400	68

This relation does not always hold for the reason that a gram one kind of dirt may contain infinitely more organisms than an equ amount of some other kind. The difference in the solubility of vario forms of dirt always causes apparent discrepancies in this norm relation. In the majority of cases, however, the relation shown the above examples will hold reasonably true in the case of fresh mi There is also a general relation between the number of bacteria fresh milk and the length of time it will keep before souring and cu ling. In this case the relation is in inverse ratio, the smaller the init contamination, the longer the keeping time, and *vice versa.* This lation is also shown in the table given above. There are many regularities, however, in this relation because of differences in t flora of fresh milk. It may frequently happen that a sample of m containing a relatively high number of organisms will not sour quickly as another sample with a smaller original germ content. T associative action of the different species of organisms is an import factor here. In making comparisons of this sort, it is, of cour necessary that the different samples be held at the same temperatu

Milk as a Carrier of Disease-producing Organisms

It is not the purpose of this chapter to discuss in detail the disea which may be carried by milk, but a chapter on bacteriology of m would be incomplete without a brief discussion of this import subject.

From the standpoint of their relation to the health of the c sumer the microörganisms in milk may be divided into three gro on the basis of whether they are beneficial, inert or injurious to hea

Acid Forms.—The preservative properties of sour milk have b known since very ancient times. Its use as a preservative for m eggs and other perishable food products demonstrates the value sour milk as a means of preventing decomposition. It has also b known for a long time that sour milk has a certain therapeutic va because of the action of the lactic bacteria in preventing harm fermentations in the digestive tract. More recently the work Metchnikoff has shown the usefulness of sour milk both for the tr ment and prevention of intestinal disorders by inhibiting the devel ment of the putrefactive bacteria in the digestive tract. In view the value of sour milk for preventing certain forms of disease and

hibiting action on certain undesirable organisms the *Bact. lactis idi* type of bacteria must be regarded as beneficial organisms, and om the standpoint of the health of the consumer their presence in the ilk is to be welcomed rather than discouraged. As the value of ur milk drinks becomes better known the importance of this group milk bacteria will be more fully recognized.

Neutral or Inert Forms.—In ordinary milk there is a large class of cteria which, so far as known, have no appreciable effect either on the composition of the milk or the health of the persons consuming This group includes a number of species, many of them being ccus forms, some of them appearing in plate cultures as chromogenic lonies. They grow more or less freely in milk, depending upon the nditions, but they are usually held in check by the acid-forming bacria and do not constitute a very important part of the flora of normal ilk. They are, therefore, of little significance from the practical andpoint except as they indicate the conditions under which the milk as been produced and handled.

Injurious Organisms.—The diseases which may be carried by milk e of two classes.

Epidemic Diseases.—The human diseases most commonly carried y milk are typhoid fever, diphtheria and scarlet fever and occasionally her diseases such as septic sore-throat, cholera and foot-and-mouth isease. The first three are by far the most important of this group. he outbreaks of typhoid fever which are traceable to milk occur ost frequently. There is a large accumulation of data showing the ccurrence of epidemics caused by infected milk. An epidemic caused y the milk supply has certain characteristics which distinguish it om epidemics resulting from other causes. A considerable number f cases of the particular disease will appear almost simultaneously nd will be distributed along some particular milk route. Usually he epidemic stops as suddenly as it began except for a few secondary ases contracted from those first taken. The source of the disease rganisms is a human patient suffering from the disease. The infection f the milk may be direct, as when a sick person handles a milk, r it may be indirect as when a person caring for a patient also works bout the milk. In other cases it may be caused by contamination f the water used in washing the utensils or by cows wading in water f infected streams and getting the organisms on their body whence

they fall into the milk pail at milking time. The return of milk bottle from the sick room sometimes is the means of infecting the milk supply

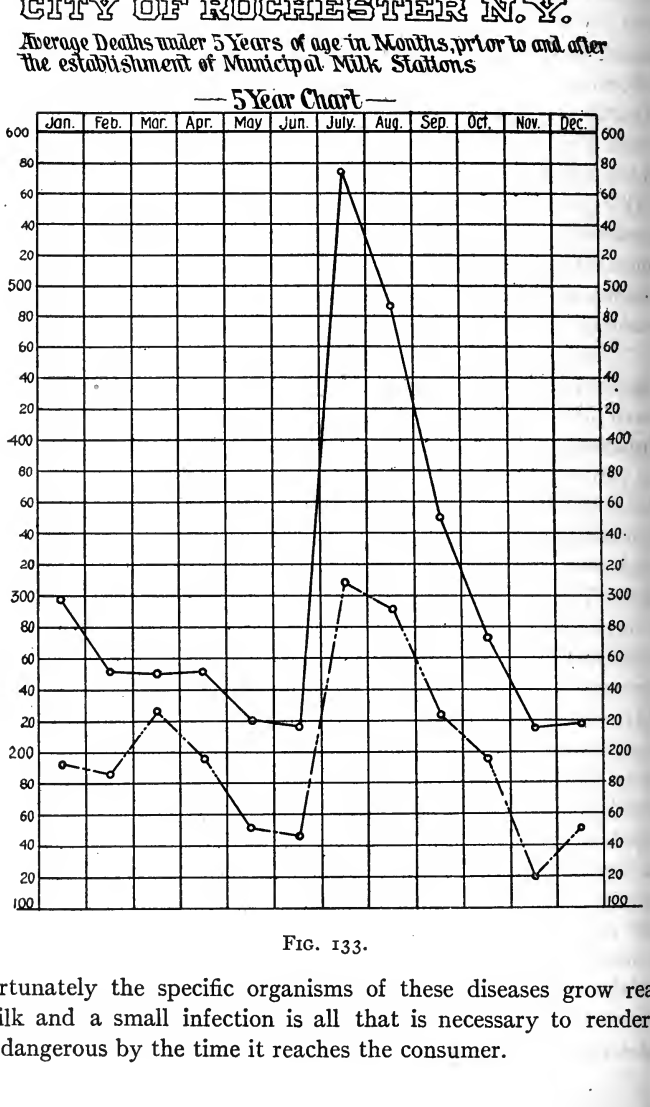


FIG. 133.

Unfortunately the specific organisms of these diseases grow readi in milk and a small infection is all that is necessary to render t milk dangerous by the time it reaches the consumer.

Non-epidemic Diseases.—There is another class of diseases which ay be carried by milk which are not characterized by a sudden out-eak, and for this reason are not so readily recognized as being asso-ated with the milk supply. One of these diseases, namely tuber-losis, is caused by the specific, well-known organism, *Bact. tuberculo-s*, which may get into the milk from the udder of a tuberculous cow by the organisms which have been given off from the digestive act of the animal becoming scattered about the stable and finally tting into the milk with particles of dust and filth. In some ses the milk may become infected by persons having the disease ing permitted to handle the milk. Fortunately for mankind *Bact. berculosis* does not multiply in milk.

Regarding the danger of contracting tuberculosis from the use of ilk there is at present some difference of opinion, but the con-nsus of opinion at the present time seems to be that there may ot be very great danger for healthy adults, but that a considerable rcentage of the cases of tuberculosis of children may be traced from e milk supply. Fortunately the temperatures used in the process pasteurization by the "holding" method are sufficient to destroy ıy of the disease bacteria known to be carried by milk.

There is another class of disorders not so well defined as the above ıt which are nevertheless of great importance from the standpoint of ıblic health, especially of young children and also to some extent of dults. This group includes such disorders as infantile diarrhœa, sum-er complaint, cholera infantum and other disorders of the digestive act. The organisms producing these troubles doubtless belong to the roup of putrefactive bacteria which come from filth. Some of the gas roducers and some of the peptonizers are probably responsible for ıese troubles. Shiga isolated from a large number of cases of infant iarrhœa a bacterium which he named *Bact. dysenteriæ*, but in general ıe specific organisms responsible for these intestinal troubles are not ell known. Their importance, however, is shown by the relation of ıe germ content of milk to infant mortality (see Fig. 133).

Bacteriological Analysis of Milk

The development of our knowledge of the relation of bacteria to the holesomeness of foods has led to a study of the bacterial content of

milk as a means of determining its purity. The methods used for th purpose have followed quite closely those of the water bacteriologists.

For many years, dairy bacteriologists have endeavored to determine the numb of organisms in milk by plating it into nutrient agar or gelatin. By this meth the number of colonies developing in the plates is assumed to represent the ger content of the milk. But even when the best methods are employed, the plate cou represents only the approximate and not the exact number of bacteria in any lot milk. It should also be borne in mind that such counts are always underestimate because of the fact that not all species will develop in any given medium or incubatic temperature. The careful worker can recognize certain types of bacteria in pla media, but the addition of blue litmus solution to either agar or gelatin, great assists in the differentiation of types and species.

THE DIRECT MICROSCOPIC METHOD.—The plating method is expensive becau of the large amount of time and materials needed. It is not possible for one pers to handle a large number of samples at one time. In routine work in the city labor tories this labor has been a serious drawback to this method. In order to decrea the labor and give greater possibilities to the work Stewart devised a method l which the bacterial condition of milk can be studied by direct microscopic examin tion. His purpose was to determine only the species present, but later Slack a still more recently Breed developed the method for determining the approxima numbers as well as the general species present in a given sample of milk.

LEUCOCYTES.—The microscopic examination of milk sediment revealed the fa that frequently a sample would be found which showed the presence of leucocytes greater or less numbers. The presence of these cells was regarded as importa because it was assumed that they showed the presence of inflammation and pus fc mation in the udders of the cows producing the milk.

Several methods have been used for determining the leucocyte content of mil "The Smear Sediment" and "Blood Counter" are methods which more strict belong to laboratory practices and will not be considered in this place.

BACTERIOLOGICAL MILK STANDARDS

The relation of the bacterial content of milk to its wholesomene has led to the adoption of certain standards by the boards of health our cities. These standards recognize the fact that the germ content milk in the large cities is greater than in the smaller ones because of th greater distance from which it is shipped and its age on arrival to th city. New York City in 1900 adopted a maximum limit of 1,000,0 per c.c. Later Boston established a limit of 500,000 Chicago 1,000,0 from May to September, inclusive, and 500,000 from October to Apr inclusive and Rochester 100,000. Other cities have made simil standards.

Stokes' standard for the number of leucocytes permissible in normal milk was 5 per field of the 1/12 objective in his smeared sediment preparation. Bergey found so many samples running above this number that he made the limit 10 cells per field and felt that no milk containing more than this number should be used for food. Later Slack raised the limit to 50 cells per field. The reason for changing the standard was due partly to the larger numbers found as a result of improved methods but more especially to the discovery that milk from apparently healthy cows normally contains leucocytes in excess of the first standards set.

With the development of the dairy score card, there was a decided tendency to place emphasis on the sanitary conditions at the farm rather than on the germ content of the milk. But it was soon discovered that the farm score did not necessarily show the true condition of the milk, and at present, the tendency seems to be toward placing more confidence in the germ content as the best measure of the true conditions of production and handling. However, the fact must be recognized that our methods of bacteriological analysis are not sufficiently accurate to justify the bacteriologist in passing judgment concerning the quality of any milk supply on a single analysis. In order to secure results which are at all trustworthy, a *series* of analyses must be considered.

It is held by some that a numerical standard is of little value since the actual number of organisms present in a given lot of milk may not be a correct measure of its wholesomeness. For this reason some cities pay little attention to the numbers of bacteria present but base their standards wholly on the species and the quality of the milk is judged on the presence and numbers of streptococci, *B. coli*, leucocytes, sediment. Milk is passed or condemned on the basis of any one or combination of these conditions.

In recent years there has been a tendency to combine these two standards using the total germ content as a measure of the care the milk has had and the presence or absence of certain groups or species as an indication of the occurrence of pathological conditions in the cows producing the milk. The practice in most city laboratories now is to make use of both the numbers and the species present in determining the quality of the milk supply.

Value of Bacteriological Milk Standards and Analyses

Regarding the value of bacteriological standards for milk there i still some difference of opinion among milk bacteriologists. The gerr content of any lot of milk is largely dependent upon three factors: th number of organisms getting into the fresh milk; the temperature a which it is kept; the age of the milk when analysis is made.

The high bacterial count in any lot of milk may be the result of an one of these conditions or a combination of them. A high count mear that there has been carelessness either in the production, resulting i high initial contamination, or in the subsequent handling permitting rapid multiplication of the organisms, or that the milk is old.

On the other hand, milk with a low germ content can be obtaine only where the original contamination is small and the milk has bee held at low temperatures. A low count, therefore, means care both i the production and later handling of the milk.

While the germ content may be regarded as a general index to th care the milk has received, it may not at all indicate its wholesomenes A high count may be the result of the rapid growth of the lactic bacteri in which case the milk may be perfectly safe and wholesome. On th other hand, the count may be quite small but contain pathogenic specie The bacteria count is valuable as showing the sanitary conditions production and handling, but much care should be used in the inte pretation of such results. In some ways a direct microscopic examin tion of the milk sediment is much more satisfactory. The skille analyst can recognize certain types which may indicate the sanita quality of the milk. With sufficient experience one can recognize strej tococci, certain other groups and leucocytes. The presence and abu dance of one or more of these groups may indicate the nature of th original contamination and the existence of diseases in the udders cows. If rightly interpreted the information thus obtained is of muc value. The weakness of this method lies in the fact that it is not alwa possible to recognize the above types of organisms. In a smear prep ration it is not possible to differentiate between pathogenic and no pathogenic streptococci or between *B. coli* and certain other type The presence of unusual numbers of streptococci and pus cells ma indicate the existence of disease in the cows and when this condition found in the milk it is often possible to trace it back to the farm and l

e the diseased cow and prevent her milk from being used for human
sumption.

The tendency at present is to combine the quantitative and quali-
ive analyses and the results thus obtained in the hands of the careful
rker are of much practical value in controlling the quality of a city's
k supply.

CHAPTER II*

THE RELATION OF MICROORGANISMS TO BUTTER

Butter is the fat of milk that has been largely freed from the othe constituents of milk by the processes of creaming and churning If milk is allowed to stand, the fat, which is in the form of minut globules, accumulates in the upper layers of the milk because its spe cific gravity is much lower than that of milk serum. In modern prac tice the fat is concentrated in a portion of the milk by passing the mil through a cream separator. In the rapidly revolving bowl of the separa tor the centrifugal force exerted is many times greater than that of grav ity and the fat is rapidly and efficiently removed. The cream, which i obtained by these methods, contains varying amounts of fat whic is further concentrated, by subjecting it to agitation in the churnin process. The globules of fat cohere to form larger and larger masse until the entire amount of fat is brought into a single mass, the butte

Types of Butter

Sweet-cream Butter.—If little or no increase in the acidity c the milk or cream develops, previous to churning, the butter will hav certain marked characteristics and is called *sweet cream* butter. I is especially characterized by its low flavor, since it has only th flavor of the fat of milk which is not marked. This is usually known a the *primary* flavor of butter. Sweet-cream butter is also marked b the rapidity with which it undergoes decomposition changes, especiall when it is made from raw cream.

Sour-cream Butter.—If the cream is allowed to undergo the aci fermentation, the butter will differ markedly both in degree and kin of flavor from that prepared from sweet cream, and as a rule its keepin qualities are much better than those of sweet-cream butter. This typ of butter is made throughout northern Europe, England and he

* Prepared by E. G. Hastings.

co nies, and in America. It may be said to be the standard butter of he world since it is the type made in all the great dairy countries. Sweet-cream butter is made especially in southern Europe, and in limited amounts in other countries.

The intensity and kind of flavor of butter is thus dependent on the acid fermentation of the milk or cream. It is not believed that the fat undergoes any changes during the acid fermentation of the milk which could produce the flavor of sour-cream butter, but rather that the increase in flavor is due to the absorption by the butter fat of certain of he compounds formed in the acid fermentation. It is not essential that the fat be present during the acid fermentation in order to impart flavor to the butter. If sweet cream is mixed with sour milk and churned at once, the flavoring compounds are absorbed by the fat from the fermented milk, and the butter will have much the same flavor, both as to intensity and kind, as though the fat had been present during the fermentation. The churning of a mixture of sweet cream and sour milk is used commercially and is identical with the methods employed by the manufacturers of oleomargarine and renovated butter to impart flavor to the flavorless fats they employ. It is impossible to recognize these substitutes for butter by their flavor since it is identical with and derived from the same source as the flavor of butter.

In the past many ideas have been expressed as to the source of the flavor of butter; some have asserted that it is due, in part, to the decomposition of the proteins of milk by proteolytic bacteria. Both practical experience and experimental work have demonstrated the connection between the acid fermentation of milk and the flavor of butter, and it is certain that what is now considered the finest type of butter can be made from cream in which only acid-forming bacteria (see Chap. I) have grown.

Flavor of Butter

Control of Butter Flavor.—The commercial value of any sample of butter is largely determined by its flavor. If it is lacking in flavor and aroma, or if it has a poor flavor, it brings a low price. The importance of being able to control the flavor, both as to degree and kind, in the manufacture of butter has increased greatly in recent

years, because of the introduction of the creamery system, which h largely supplanted the making of butter on the farm. The financi success of any creamery is largely dependent upon the ability of t butter maker to control the flavor of the product, so that it shall l uniform from day to day. It is asserted that one of the facto in the remarkable invasion of Denmark into the butter markets the world is the uniformity of the Danish butter, not only from a sing creamery, but from all the creameries of the country. To the Dan we owe the most improved methods for the control of the flavor butter.

The other points, texture, color and salt, which the judge of butt takes into consideration, can be easily controlled, since they are due mechanical operations. The flavor, on the other hand, is due to the b products which are formed by microörganisms in the fermentation of t milk and cream, and which are absorbed and held by the fat. If any the products formed possesses a disagreeable taste or an offensive odo the flavor and aroma of the butter will be impaired. It is thus evide that the control of the flavor of butter is dependent on the control of t acid-forming bacteria that ferment the milk and cream. This is the pro lem of the modern butter-maker and the modern methods seek to gi him this control, to enable him to eliminate the undesirable bacteria, *coli* and *Bact. aerogenes*, the second group,* and to insure the predon nance of the desirable bacteria, *Bact. lactis acidi*. This gene statement is not to be interpreted as meaning that all bacteria th injure the flavor of butter are to be included in the group mentione for many other types of bacteria, when present in milk in large numbe may injure the flavor of the butter prepared from it.

The acid fermentation of the cream is most frequently called t ripening of cream and sour-cream butter is frequently called ripene cream butter. The ripening of the cream not only increases the flav of the product, but it enhances its keeping quality. The ripening of t cream also aids in the mechanical process of churning, the sour crea churning more easily and with less loss of fat in the butter milk.

KINDS AND NUMBERS OF BACTERIA IN CREAM.—The number a kinds of bacteria found in cream are dependent upon the number a kind in the milk from which the cream is obtained. The cream wi however, contain a greater number of bacteria per unit volume than t

* See Chap. I, Div. IV, in which the groups of bacteria are considered.

nk, since the immense number of fat globules passing through the nk serum carry mechanically a considerable proportion of the bacteria o he milk into the cream. This phenomenon is to be noted in gravity c aming, but to a much greater extent in the removal of the cream by u of the separator.

Spontaneous Ripening of Cream.—By this expression is meant t fermentation of the cream by those acid-forming bacteria that have, f m one source and another, gained entrance to it, but which have not b n intentionally added. Under these conditions the butter-maker can e rt but little control over the fermentation. A very considerable part o he butter made from such cream has an excellent flavor, because at t temperature at which cream is usually kept, *Bact. lactis acidi* and r ated organisms are the primary factors concerned in its fermentation a l their by-products produce desirable flavors in butter. It has often b n asserted that the highest type of butter can be made only from s ntaneously ripened cream.

As the cream from many farms was assembled at a creamery for the r nufacture of butter, it became evident that some means of controlling t type of fermentation in the cream was needed. If the milk had been p duced under clean conditions, and had been received at the creamery b fore the acid fermentation had gone on to any extent, and if the c am was then kept at temperatures most favorable for the lactic bac- t ia, the product was likely to be of good quality, but such ideal condi- t ns did not always obtain. Cream containing a large proportion of h rmful bacteria, or in an advanced state of fermentation, or possessing a undesirable flavor was often received, and the butter-maker could n t control the quality of the product under such conditions.

Use of Cultures in Butter Making.—As the science of micro- b logy progressed and the rôle of microörganisms in all kinds of fermen- tion became known, it was evident that the control of the causal or- nism is an important factor in determining the quality of any product the fermentation industries. In the manufacture of butter, the first st p in this direction was the addition of some fermented milk, cream, or buttermilk to the cream to be ripened. In this manner the number acid-forming organisms in the cream was greatly increased, and the f rmentation went on more rapidly and in a more definite direction than w thout such additions, as the bacteria added were largely of the d sirable group, *Bact. lactis acidi.* The addition of fermented milk to

accelerate the souring of cream antedates by many hundred years th science of bacteriology.

The next logical step in the development of the process was the use c the same types of bacteria from day to day. Cultures of these were ob tained by allowing a quantity of milk to sour, and if it had the desire flavor, a small amount of it was added to another quantity of milk tha had been heated, in order to destroy the acid-forming bacteria it co tained. By the daily preparation of some heated milk, and the inocula tion of it with the soured milk previously prepared, the butter-mak could use the same types of bacteria for an indefinite time for additio to the cream.

It had been found by Hansen that, in order to control the flavor beer, pure cultures of yeasts must be used for the fermentation of th wort. The success of this method in the brewing industry led to th introduction of pure lactic cultures for the fermentation of cream. Th use of such cultures was suggested independently by Storch, a Danis bacteriologist and by Weigmann, the director of the dairy experimen station at Kiel in Germany, in 1890. Many cultures were isolated an tested as to their effect on the flavor of butter. Those found to h desirable could be maintained in the laboratory, and could be furnishe to butter-makers to be used and propagated in a manner similar to th method employed with the impure and less constant home-made starter The pure cultures of lactic bacteria are widely used at present in th butter-producing countries of the world and their use is being constantl extended, as butter makers come to recognize the importance of co trolling the ripening of cream.

It was found that the butter made from cream ripened by pure lact cultures did not possess as high a flavor as did the finest butter mad from naturally ripened cream. This led to the search for organism that could be used alone, or together, with the lactic bacteria, and whic should give the high flavor desired. Such cultures were found, bu their use did not prove practical, either because they did not maintai their properties on continued cultivation, or because of their effect c the keeping quality of the product. The difference in flavor in the ca of butter made from naturally ripened cream and that from cream rip ened by pure lactic cultures is undoubtedly due to the products of th *B. coli-aerogenes* group.

The acid in spontaneously soured milk is very evident to the tas

wen the acidity is 0.6 per cent and above; the volatile acids formed by tl members of the colon-aerogenes and coccus groups impart a sharp, pngent taste. In milk of like acidity fermented by pure cultures of *Bt. lactis acidi*, the acid is scarcely evident to the taste and there is no slrpness, due to the absence of volatile acids. This same difference apears in the butter made from the two kinds of milk.

The low flavor of the butter made from cream ripened by pure ctures was one of the factors that prevented the rapid introduction othe cultures in this country. The demands of the butter market hre changed and the mild flavored butter, which is now considered tbe the finest, can be made by the use of pure cultures in the fermentati of pure sweet cream.

Commercial Cultures.—In this country the preparation and dribution of cultures for the ripening of cream is largely in the hids of commercial firms; hence, the term "commercial culture" isapplied to them. The different pure cultures are propagated in tl laboratory of the maker; they are sent out either as liquid cultures, a mall mass of milk or bouillon inoculated with the organism, or in a ry form, the latter being prepared by mixing a culture of the organism wh an inert substance, such as milk sugar, milk powder, or starch, a drying at a low temperature. In a liquid the organisms are eosed to the effects of their own by-products, and the vitality of tl culture is rapidly lost. Such cultures must be used when fresh iorder to give good results, and they cannot be kept in stock by the manufacturer or dealer. The resistance of *Bact. lactis acidi* to desiccion is great; it thus lends itself to the preparation of the dry cultures, in which the organisms remain in a dormant condition and retain tlir vitality for long periods.

Most of the cultures now sold are pure, as this term is used in bactology, still others contain non-acid-forming organisms, intentionally aled or introduced accidentally during the process of preparation. Ithe lactic bacteria are present in such cultures in large numbers, tl impurities are usually of small practical significance. In the past scalled "duplex" cultures have been sold which were supposed to ctain an acid-forming organism and a second organism that was tenhance the flavor of the product. Such cultures are no longer sold.

For the propagation in the creamery the contents of the container prchased are added to a small mass of milk that has been heated

to destroy all non-spore-forming bacteria and other microörganism the milk, after being inoculated, is incubated at favorable temper tures and when curdled can be used for the inoculation of the secor and larger quantity. The process of inoculating a quantity of mi is carried out daily. It is impossible for the butter-maker to propaga the culture so as to maintain the original purity, but with care in tl heating of the milk the sterilization of all utensils and the maintainir of proper temperatures, the contamination that occurs will not inju the culture for practical work. The cultures propagated under su conditions gradually deteriorate and recourse must be had soon or later to a fresh culture. The contamination that is of the greate practical significance is undoubtedly that with other acid-formi bacteria rather than with the forms that remain in the milk aft heating.

Many of the cultures gradually lose their fermentative properti and do not form acid rapidly and in sufficient amounts to insu exhaustive churning and to produce the desired degree of flavor the product. Cultures frequently become slimy or ropy on propag tion. This is not necessarily due to contamination with speci slime-forming organisms but rather to a change in the lactic organis itself. Such an abnormality usually persists for only a short peri and the conditions that govern its appearance and disappearan are not known. It is asserted by practical butter makers that t development of too high an acidity in the cultures as they are pro gated in the creameries permanently impairs the value of t culture.

The cultures are propagated in skim-milk. Where this is not ava able, unsweetened, condensed milk or milk powder have been employ Efforts have been made to grow the bacteria in some other kind medium than milk, but without success. The starter is said to ripe or in the best condition for use soon after curdling, or when t acidity is 0.5 to 0.7 per cent, as at this time it contains the maxim number of living cells. The practical man thus uses the curdl as an indication of the ripeness of the starter. The curdled milk sho show no free whey, and the curd should be easily broken up to fo a creamy mass that can be uniformly incorporated with the crea The temperature of incubation and the amount of initial inoculat determine the rapidity with which the acid fermentation will progr

the maker seeking to regulate these so that the culture shall be ripe at the desired time each day.

USE OF PURE CULTURES IN RAW CREAM.—The cream as it reaches the creamery contains a greater or less number of acid-forming bacteria that ultimately will cause it to ripen and the flavor of the butter will be due to the by-products of the mixture of bacteria. If, through the addition of a pure culture, the relative number of organisms that are known to be favorable is greatly increased, the flavor of the product should be improved. This has been found to be true in practice and it is now believed that pure cultures are of value not only in the ripening of sweet cream, but that the addition of a relatively large amount of starter to cream that is already fermented will enhance the value of the butter.

USE OF PURE CULTURES IN PASTEURIZED CREAM.—It is evident that the maker has but imperfect control over the fermentative processes when raw cream is treated with a pure culture. To insure more perfect control the destruction of the contained bacteria and the subsequent inoculation of the cream with a pure culture is indicated. The introduction of the process of pasteurization of cream for butter making was due to Storch. In Denmark this method is used almost exclusively. It has been introduced into the other dairy countries of the world and is constantly spreading. Pasteurization combined with the use of the pure culture represents the highest type of modern butter making, and where the raw product can be obtained in a fresh condition the butter-maker has perfect control over the bacteria that cause the ripening; hence he can control the flavor of the butter, both qualitatively and quantitatively.

The intensity of flavor of butter is dependent upon the amount of acid that is developed in the cream or more correctly on the ratio between the amount of fat and the by-products of the acid fermentation. If these by-products are small in amount, as in cream having a low acidity, the flavor of the butter will be low. If the acidity is allowed to reach the maximum, the flavor will be much higher. Thus the maker can control the intensity of flavor of butter as accurately as he can the kind of flavor. With rich cream, the acidity that can be developed is small and the ratio between the fat and the products of fermentation is low; thus, the flavor of butter made from very heavy cream is certain to be low.

Pure Cultures in Oleomargarine and Renovated Butter.—It was previously mentioned that the manufacturer of butter substitutes employs the same methods to impart butter flavor to his products as does the butter-maker. The oleomargarine manufacturers employ pure cultures of lactic bacteria for the fermenting of milk that is mixed with the fats they employ. The same practice is followed by the manufacturer of renovated butter. Many of the creameries of the western states receive cream that is shipped long distances and is collected from the farms but once or twice a week. It is thus in an advanced state of fermentation when it reaches the creamery. In order to prepare from this grade of cream, which often has a more undesirable flavor, a merchantable product, various means are employed to remove the flavoring substances and to replace them with desirable flavors from the pure cultures. The acidity may be reduced by the addition of lime so that the cream can be pasteurized; the cream may be aerated by passing air through it, or it may be mixed with water and reseparated. After such treatment it is mixed with a large proportion of milk fermented by a pure culture and churned. The resulting product is constantly sold as the highest grade of creamery butter.

Abnormal Flavors of Butter.—Most of the abnormal flavors of butter are traceable to the partial replacement of the desirable acid-forming bacteria with other types of microörganisms Many samples of butter having abnormal flavors have been examined, and the organisms believed to be the cause isolated and studied but it cannot be said that any particular group of microörganisms can be associated with any of the abnormal flavors met. It is asserted that "oily" butter, *i.e.*, that having the taste of machine oil, is caused by bacteria and by microörganisms that decompose the fat, as *Oidium lactis*, yeasts, and liquefying bacteria. Organisms of the *B. coli* group that produce a turnip-like flavor in butter have been described by Weigmann. The flavors of putrid butter, fishy butter and also many other abnormal flavors have been ascribed to bacteria.

Other abnormal flavors may be due to the presence in the milk of certain aromatic principles contained in the feed and excreted in the milk. Cabbage, turnips, and other plants impart their characteristic taste to the milk and butter.

Decomposition Processes in Butter

Butter is a finished product at the time it is removed from the mod- e churn and all subsequent changes are likely to cause more or less d erioration. The specific causes of these changes are not well known b it is very evident from a study of the conditions that favor or retard t l appearance of the flavors, characterizing these changes, that biolog- ic factors are concerned. When raw cream is used, sweet-cream butter h very poor keeping qualities. As the proportion of acid-forming b teria in butter is increased, either by the fermentation of the cream, b the addition of pure cultures, and through the use of the latter in c nection with pasteurization, the keeping qualities are enhanced. O the butter made from ripened cream, that prepared from cream, h dled in a clean manner, and thoroughly pasteurized and ripened with a ure culture of *Bact. lactis acidi*, has the best keeping quality. If fr h, sweet, clean cream is pasteurized, the butter will have better k ping quality than when made from the same cream pasteurized and ri ned with a pure culture. This is evidence that not only the bac- te a other than *Bact. lactis acidi* are harmful, but that this organism, th has usually been considered without influence on the keeping q lity, must be classed as one of the factors in the decomposition of b er.

It has been shown that the bacterial content of the water used for th washing of the butter has an influence on the keeping quality. If th water is of surface origin and contains the bacteria peculiar to these ty s of waters, its influence may be marked and some method of treat- m t must be followed. Filtering or heating the water has been re- so ed to, the latter with marked success. A pure water will contain so w bacteria that they will not exert any noticeable influence on the k ping quality of the butter.

Storage temperature also has a marked influence on the deterioration ch ges in butter. Modern butter-storage rooms are kept below 0°; the butter deteriorates slowly during storage at these tempera- tu s, but on removal undergoes change much more rapidly than w ld have been true before storage. Another factor that is of influence in e keeping of butter is the amount of salt used. In salted butter, the co ained water is a concentrated brine; in such a medium most forms of acteria are unable to grow. Small packages deteriorate more

rapidly than large ones, because the proportion of the mass of butt exposed to the air is relatively greater. Exposure to light is al claimed to exert a harmful influence. Antiseptic substances such borax and boric acid have a marked effect on the deterioration chang The New Zealand and Australian butter exported to the English ma kets is treated with preservatives.

A large amount of experimental work has been done in order determine the effect of specific organisms on the keeping quality butter. The results obtained have not been definite and it is not certa that the organisms employed are constantly concerned in the deterio tion changes. It is very probable that both bacteria and molds exe an influence. The chemical changes that take place in the spoiling butter are no better known than are the causal factors. It has be asserted that there is a decomposition of the glycerides with a resulti increase in free acids. It has been shown that this does not alwa occur; that a butter may be in an advanced state of decomposition a its content in volatile acids not be higher than when fresh. Two typ of changes are usually distinguished, rancidity and the appearance o tallow-like odor. The latter may be due to purely chemical facto while the former is quite certainly biological.

Moldy butter is a frequent trouble encountered by the butter-mak If the butter is not salted, molds may develop just below the surfa The most usual form of mold to appear is one with black hyphæ; t slightest development of which will be evident on the butter. In t case of salted butter, mold on the butter itself is very rare, due app ently to the concentration of the brine in the butter. The parchme paper in which print butter is wrapped and with which the butter co tainers are lined is an excellent substratum for mold growth. If t papers and containers are badly contaminated with mold spores, they have been kept under such conditions as to permit of a limit amount of growth before they are used, the development of the mold the paper after it is brought into contact with the butter is likely to rapid, even at low temperatures, and the butter is likely to reach t market in an objectionable condition. The paper may be render free from molds by placing it in water which has been heated to at le 80°. Butter tubs are scalded, steamed, or soaked in brine or treat with a dilute solution of formalin in order to destroy the mold spo that may be present. The most efficient manner of preventing troul

to coat the inside of the butter tub with paraffin. This prevents ouble from the container but not from the paper.

Pathogenic Bacteria in Butter

If the milk contains pathogenic bacteria, they are certain to pass to the cream and be incorporated in the butter. It is not believed at butter is an important agent in the distribution of the organisms tuberculosis and typhoid fever, although both are able to exist in lted butter for over two months. Foot-and-mouth disease is said caused in humans by the use of butter made from the milk of fected animals, but this may still be regarded as a mooted uestion.

CHAPTER III*

THE RELATION OF MICROORGANISMS TO CHEESE

General

Cheese consists of the fat and casein of milk, together with th insoluble salts; however, along with these constituents are carrie some of the moisture of milk, in which are dissolved small quantitie of sugar, albumin, and salts. The amount of moisture and solubl constituents found in cheese is determined by the amount of whe incorporated in the curd.

In the process of making cheese, it is necessary to curdle the milk thus enabling the separation of the casein and fat from the milk serum Two methods are employed to accomplish this purpose, and, as a result, two types of cheeses are produced.

Types of Cheese

These types may be designated as "*Acid-curd Cheeses*" and "*Rennet curd Cheeses.*"

Acid-curd Cheeses.—The curdling may be accomplished by allowing the milk to undergo acid fermentation, either spontaneously through the action of the normal flora of the milk, or through th addition of pure lactic cultures. Most acid-curd cheeses are ready for use as soon as the whey has been removed by draining and th curds salted. Acid-curd cheeses are not commercially important They are made for local consumption and are to be classed as a form of sour milk. They owe their flavor to the products of the acid fermentation, especially lactic acid. The moisture content is high which, together with the acid reaction, favors the growth of molds and yeasts. These biological agents may soon spoil the cheese.

Rennet-curd Cheeses.—All of the important varieties of cheese are made by the use of rennet for the curdling of the milk. Ove

* Prepared by E. G. Hastings.

our hundred kinds of rennet-curd cheeses are made, but only twelve o fifteen are of great commercial importance. With few exceptions, hey are made from cow's milk. From the same raw material—milk, ennet, and salt—therefore, a wide variety of products, differing n texture, taste and odor, is obtained. This fact indicates the im-ortance of biological factors in the changes the curd undergoes during he ripening process.

The rennet-curd cheeses may be divided into: (1) *hard cheeses;* 2) *soft cheeses;* the initial difference is largely in the amount of whey ft in the curd during the making of the cheese. The two great groups rennet-curd cheeses gradually merge into each other in varieties at by some are classed as hard cheese, by others as soft cheese.

The rennet-curd cheeses, as a rule, are at first tough and rubber-like texture. The curd, which is not easily digested, is quite in soluble water and is devoid of flavor and aroma. The curd must pass rough a complete series of chemical and physical changes, which ter its texture, solubility, and digestibility, and give to it a flavor d aroma by which the different kinds of rennet-curd cheeses are pecially to be differentiated.

In the hard cheeses the factors concerned in these changes act in a iform manner throughout the entire mass of the cheese, making possible to manufacture such cheeses in any desired size. In the se of the soft cheeses, the ripening changes are largely due to agents ich grow only on the surface; the products of such agents by means diffusion gradually affect the entire mass. In order that this may e place within a reasonable time, it is essential that these cheeses made in small sizes. Then, too, the soft texture of such cheeses kes it impossible to handle them commercially in large sizes.

Conditions Affecting the Making of Cheese

Quality of Milk.—In the curdling of milk by rennet the solid blies present in the milk are retained in the curd, thus the fat gbules are held, as are also the bacteria. The latter continue to gw as they would have done in the milk except that growth st take place in the form of colonies as in the solid culture media o the bacteriologist. The bacteria, however, produce the same fer-ntation in the curd as they would have done in the uncurdled milk.

The butter-maker can control, through pasteurization and the us of pure lactic cultures, the fermentation of the cream. The pasteur zation may be so efficient as to destroy all non-spore-forming bacteri since the quality of the product will not be impaired by the use temperatures approximating the boiling point. The cheese-mak

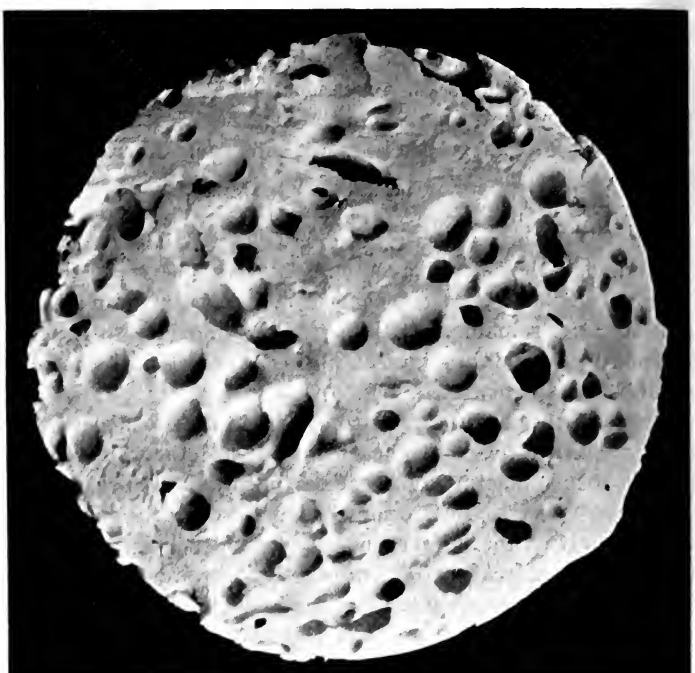


FIG. 134.—The type of curd obtained from milk in which the acid-forming fl consists largely of organisms of the *B. coli-aerogenes* group. Many gas holes a few irregular shaped, angular, mechanical holes due to imperfect "mattin (*Original.*)

is much more dependent on the original quality of the milk, since has not been found possible to make most of the important varieties cheeses from pasteurized milk. If undesirable forms of microörganis are present in the milk, they will pass into the cheese and there produ their harmful effects. Through the addition of pure cultures

act. lactis acidi to the milk, the proportion of desirable bacteria can increased and a partial control of the fermentation thus secured.

TESTS FOR THE QUALITY OF MILK.—Methods by which the cheese aker can determine, in a rough manner, the kinds of bacteria present

FIG. 135.—The type of curd obtained from milk in which the acid forming flora sists almost wholly of *Bact. lactis acidi*. No gas holes and no marked mechanical les as the curd has "matted" almost perfectly. (*Original.*)

ve been devised. The bacteria most dreaded and most frequently esent are those of the *B. coli-aerogenes* group.

The method most frequently used for their detection consists in ubating a sample of the milk to be tested at temperatures ranging m 35° to 40° for a few hours and noting the type of curd that is rmed. Milk suitable for cheese making should show the solid curd aracteristic of the *Bact. lactis acidi* group, while gassy curds or soft

and partially digested curds are indicative of bacteria that are likely be harmful in the cheese.

An improvement over the fermentation test of foreign origin h been devised by Babcock and Russell and is known as the *Wiscons Curd Test.* It has for its basis the same principle as the simple ferme tation test; however, a modification is introduced; the milk is curdled b the addition of rennet and the curd is cut and drained to free it from tl whey as completely as possible.

The undesirable organisms most likely to be present in milk a those of the *B. coli-aerogenes* group; therefore the jars containing tl curds should be kept at temperatures, 35° to 40°, that will favor the development. The great advantage of the *Wisconsin Curd Test* is i greater delicacy, since the bacteria are concentrated in a small volum and thus their presence is more evident than would be the case in tl larger mass of curd obtained when no rennet is added. The curd ca also be removed from the jar, cut, tasted, and its texture determined, a of which aid in judging the quality of the milk. The curd should ha a clean acid odor and taste; it should be free from sliminess on the su face, and possess a uniform texture. Such a curd can be obtained on in the presence of a considerable number of lactic bacteria. Ve clean, fresh milk is likely to give an undesirable result, since mi always contains microörganisms which will grow rapidly at the hig temperature in the absence of the acid-forming bacteria and which w usually produce undesirable flavors in the curd. This fact should b kept in mind in the testing of market milk.

Ripening of Milk.*—The methods for the determination of acidit in milk have very considerable limits of error. It is not possible t detect any increase in acidity until the number of acid-forming bacter has increased to hundreds of thousands per c.c. Originally it wa thought that no acid was produced by the growth of the acid-formin bacteria during the initial stages of their development. This perio during which bacterial proliferation was taking place, but without a apparent increase in acidity, was known as the "period of incubation. It is now certain that this rests upon our inability to detect sma

* In order to illustrate the rôle of microörganisms in the making and ripening of cheeses, somewhat detailed summary of the present knowledge concerning their action in Cheddar chee will be given. Many of the factors concerned in the ripening of this kind of cheese also functi in the ripening of other rennet cheeses. In their description only such additional factors ne be considered as are not active in Cheddar cheese.

increases in acidity. The Cheddar cheese-maker desires milk that shall contain such a number of acid-forming bacteria that during the operations that are carried on in the first part of the cheese-making process large amounts of acid shall be formed in the curd. He thus wishes to know, as accurately as can be determined under the conditions found in the factory, the number of bacteria in the milk which he is to use. This information is gained either by the titration of the milk with a standard alkali solution or by determining the time required for the curdling of a definite quantity of milk at a definite temperature by a known quantity of rennet. Very much smaller increases in acidity can be detected by the so-called rennet test than by titrating the milk. If the milk shows the desired acidity when it reaches the factory, the making process is immediately begun. If the milk is too sweet, or in other words, too low in its bacterial content, bacterial growth is favored by warming the milk to temperatures most favorable for the lactic bacteria, 30° to 32°, and by the addition of pure cultures of *Bact. lactis acidi* which are identical in nature and the method of propagation with those used in butter making. The development of a slight acidity is known as the "ripening" of milk.

In order to insure proper rennet action the maker of Cheddar cheese desires the milk to have an acidity of about 0.2 per cent. He thus wishes milk that has passed through the period of incubation and in which the acidity has begun to increase.

Curdling of Milk.—Under the influence of a favorable temperature and the slight acidity, the milk is quickly changed by the rennin* to a firm, jelly-like mass that is cut, with appropriate knives, into small cubes. The curd encloses over 80 per cent of the bacteria in the milk. The same factors that favor the curdling of the milk favor the shrinking of the curd and the expulsion of the whey from the cubes. The development of acid within the curd is rapid, due to the concentration of large numbers of bacteria in a small volume and to the favorable environment. During the six to eight hours that elapse between the curdling of the milk and the pressing of the curd, the increase of acidity is over 0.1 per cent per hour. The following table gives the acidity of milk and the whey expressed from the curd at various stages in the making of a typical Cheddar cheese.

*The rennet used in cheese-making is obtained by extracting the abomasum, the true digestive stomach of the calf, with a solution of sodium chloride. The extract contains two enzymes a clotting or curdling enzyme, *rennin*, and a proteolytic enzyme, *pepsin*.

Acidity of milk before adding rennet.....	0.20–0.21 per cent.
Acidity of whey immediately after cutting curd.	0.14–0.145 per cent.
Acidity of whey when removed from the curd..	0.16–0.18 per cent.
Acidity of whey when curd is packed.....	0.24–0.30 per cent.
Acidity of whey when curd is milled.....	0.65–0.75 per cent.
Acidity of whey when curd is salted.....	0.90–1.10 per cent.

MANIPULATION OF THE CURD.*—The curd particles at first sho little tendency to cohere; but, as the acidity increases, the nature of t curd changes, and, when the whey is removed, the pieces of curd so cohere and ultimately form a single mass in which the original cubes curd cannot be detected. The fusion of the curd particles is known "matting" and is an important step in the Cheddar process. The la of acid formation within the curd prevents matting while the curd is the vat, and may even render difficult the fusion of the particles und pressure. The nature of the change which the curd undergoes at th stage in the manufacture is not well understood, but probably is due a combination between the paracasein and the lactic acid, the resulti compounds differing from the paracasein in physical properties and solubilities.

RIPENING OF CHEESE.—Cheese in ripening undergoes profou physical and chemical changes under the influence of a number factors, which for purposes of discussion may be divided into two grou those by which the content of soluble nitrogen in the cheese is increas and the digestibility enhanced; and those which cause the formation flavoring substances. During the ripening of the cheese the mal can do little toward the control of the factors which ultimately det mine its commercial value. As in butter, the flavor is the most imp tant characteristic of the ripened cheese and the most difficult control.

Theories of Cheese Ripening.—Many theories have been advanc to explain the changes that occur during the ripening process. Ducla a French microbiologist, studied the bacterial flora of Cantal che by aid of the crude methods available before the introduction of gelatin-plate method. By the use of the dilution method, usi bouillon as the nutrient medium, he isolated a number of kinds spore-forming bacteria. The organisms formed two enzymes, a curdling enzyme related to rennin, the other a proteolytic enzy

* Cheddar cheese.

which was given the name *casease*. A chemical study of the by-products of the organisms, when growing in milk, revealed a number of compounds that had previously been found in ripe cheese, such as leucin, tyrosin, and the ammonia salts of acetic, valeric and carbonic acids. The cultures often possessed a cheese-like odor. These facts led Duclaux to believe this class of organisms were responsible for the ripening of the hard cheese in question. The generic name *Tyrothrix* was applied on account of the supposed relation to cheese. This term is still found in current bacteriological literature. The methods employed by Duclaux were such as favored the growth of the liquefying, rather than the acid-forming bacteria. To the latter more recent investigators have devoted attention.

The theory that the proteolytic bacteria function in the ripening of hard cheese has been more recently emphasized by Adametz. It is sufficient to say that the number of spore-forming proteolytic bacteria in cheese is not sufficiently large, nor is their presence so constant that any importance can be attached to them. Any agent to be considered as a factor in the ripening process must be present in every cheese in sufficient numbers to account for the change for which it is considered responsible. Such agents should be capable of demonstration. It should be remembered that, by following the rules laid down by the practical maker, a normal cheese can invariably be made, hence the factors of importance in the ripening must be constantly present in the milk or rennet. It is doubtful whether the liquefying bacteria will satisfy this requirement. It has been shown by de Freudenreich that such organisms, even when added to milk in large numbers, exert no influence on the ripening of hard cheese, since the conditions within the cheese are not such that growth can occur.

De Freudenreich, a Swiss microbiologist, by the aid of modern methods, demonstrated the constant presence of certain classes of acid-forming bacteria in Swiss cheese, and to them ascribed an important rôle in the ripening of this hard cheese. He was led to this conclusion by their great numbers in the fresh cheese, and by the fact that cheese made from milk drawn under aseptic conditions, which thus contains no lactic bacteria, do not ripen; through the discovery, also, that certain of the lactic bacteria, predominating in Swiss cheese, those of the *Bact. bulgaricum* group, exert a solvent effect on the casein of milk, although they are devoid of action on gelatin.

Babcock and Russell demonstrated the presence of an inher proteolytic enzyme in milk, to which the term *galactase* was appli This enzyme can be demonstrated by preserving a sample of fr milk with chloroform or other mild antiseptic. At 37° curdl occurs in three to four weeks; the content of soluble nitrogen in milk is slowly augmented. The presence of this proteolytic enzy together with the fact that a normal cheese cannot be made from m in which this enzyme has been destroyed by heat, led these inve gators to consider this inherent enzyme of milk an important fac in cheese ripening.

*Present Knowledge of Causal Factors.**—The rôle of certain fact in the ripening of Cheddar cheese has been established beyond do by the chemical and bacteriological investigations of recent ye It is certain that acid-forming bacteria are essential factors in ripening of this kind of hard cheese, and probably all kinds of ren cheeses.

As has been shown the growth of acid-forming bacteria is ra during the making of Cheddar cheese. The growth continues dur the pressing and subsequent thereto; the maximum number of la bacteria is found when the cheese is one to five days old. As many 1,500,000,000 per g. of the moist cheese have been demonstrat

Causes of Proteolysis.—The proteolytic action of rennet extract the paracasein of cheese was demonstrated by Babcock and Russ and by Jensen. This property is due to the fact that rennet extr also contains the enzyme *pepsin*, which for its action outside the bo requires conditions similar to those which obtain in the stoma in other words, the presence of sufficient acid to activate it. The hyd chloric acid secreted by the walls of the stomach acts as the activati agent in the body. The acidity resulting from the fermentation the sugar in the curd is sufficient to activate the pepsin. Under influence the paracasein is partially converted into soluble decompo tion products such as albumoses and peptones. In the absence acid-forming bacteria no acid is formed; consequently the pepsin d not become active and no proteolytic effect is produced. Under th conditions the curd remains tough and elastic and the solubility not increased. It is thus evident that acid-forming bacteria are ess tial factors in cheese ripening. The pepsin of the rennet extract a

*Cheddar cheese.

the galactase suffice to account for the initial proteolysis of the paracasn. Since neither of these enzymes forms ammonia, which is alwys found in ripe cheeses, some other factor must be responsible forhe production of this compound. It may owe its origin to microörgnisms not yet discovered.

revention of Putrefaction.—The various stages in the decomposition of ilk have been outlined in a previous chapter. Briefly they are as follws: The first evident change is the curdling due to the acid-forming baeria. Succeeding this, the acid, semi-solid mass or curd is a favorablsubstratum for the characteristic mold of milk, *Oidium lactis*, which

A *B*

G. 136.—Proteolytic action of *rennet extract* in the absence and in the presence of d-forming bacteria. *A*, sterile milk agar; a strip of filter-paper treated with *ren* was allowed to remain on the medium for one hour at 37°. No digestion of the cas. *B*, milk agar inoculated with *Bact. lactis acidi;* incubated for twenty-four hou at 37°, then treated as *A*. True digestion of the casein is indicated by the clea ng. (*Original.*)

soc forms a white, velvet-like layer over the surface of the milk. Like oth molds, this form can use acids as a source of energy. The acid is the oxidized to carbon dioxide and water, and thus the reaction of the mi is slowly changed until a point is reached which allows the putrefactve bacteria, that have remained dormant during the period of unfavrable environment, to develop. The curd is accordingly peptonize and putrefaction occurs. If the acid reaction is maintained thrgh the prevention of mold growth, the milk will be preserved from

the attacks of putrefactive organisms and will remain unchanged for unlimited time.

The second rôle of the acid-forming bacteria in cheese is to prot it against the putrefactive organisms that are constantly present milk and hence in cheese. The acid reaction of the cheese, due to persistence of lactic acid, or to the formation of volatile acids after initial fermentation, is sufficient to prevent the growth of the putref tive bacteria within the cheese. If the cheese is made from milk wh contains no acid-forming bacteria and few putrefactive ones, or if sugar is removed from the curd by washing it with water, the cheese not ripen since there is no acid to activate the pepsin; the curd will main in much the same condition as when it was removed from press. Cheese made from milk containing no acid-forming bacteria many putrefactive bacteria is likely to undergo putrefaction, since latter class of organisms finds conditions for growth in the absenc an acid reaction. Such a condition is rarely noted in a hard ch under normal conditions, but may be produced experimentally. biological acid may be replaced by other acids added to the curd appropriate amounts, since these will activate the pepsin and pro the cheese against the attacks of putrefactive bacteria; but it is not tain that the cheese will develop a normal flavor when lactic ac replaced by mineral acids.

Other Groups of Bacteria in Cheese.—It has been shown at the Wisconsin Experiment Station that other groups of bacteria are constantly present in Cheddar cheese. The development of certain members of the *Bact. bulgaricum* group occurs somewhat later than that of the *Bact. lactis acidi* group. It occurs largely after the sugar has disappeared. Their numbers approximate those of the *Bact. lactis acidi* group. Coccus forms are also found in great numbers in the cheese. It is probable that these two groups may be responsible for the ammonia production, since typical cultures of both groups are able to produce small amounts of ammonia in sterile milk.

Flavor Production in Cheese.—The factors that have been discussed are undoubtedly the most important ones concerned in the proteolysis of the curd, and are thus the factors concerned in the changes of texture, solubility and digestibility. The flavor, which develops during the ripening process, has been regarded as due to the proteolysis of the paracasein. A thoroughly ripened cheese contains a large amount of

mia and related compounds. It was thus natural to consider the flavor d to these simple products of protein degradation. More recently it h been discovered that the intensity of flavor does not necessarily cor-reond to the content of the cheese in these products; indeed a cheese my have a high content of nitrogen as ammonia and yet be low in fl or.

The Wisconsin Experiment Station has found that the volatile fatty a ds of Cheddar cheese increase as the ripening progresses. In the fo owing table are given the data obtained from the detailed study of a n mal Cheddar cheese.

ACIDS IN 100 G. OF DRY MATTER

	c.c. of N/10 alkali neutralized				
	3 days	42 days	3 months	5½ months	10 months
Lactic acid.....	84.09	90.28	124.00	103.70	74.10
Acetic acid.....	11.59	29.44	24.25	25.86	12.64
Propionic acid.....	0.41	2.15	3.42	1.07.	2.63
Butyric acid.....	0.73	2.17	3.50	4.82	5.45
Caproic acid.....	0.00	0.36	0.96	1.25	2.23

It will be noted that the content of the higher volatile acids, those e ecially marked in odor, continually increases. It is possible to sepa-re other volatile compounds found in cheese from the volatile fatty a ds by distilling with steam, neutralizing the distillate with an alkali a d redistilling; the second distillate will contain the alcohols and esters p sent in the cheese. Such a distillate prepared from Cheddar cheese i ound to possess the characteristic aroma of the cheese in question. Te esters it contains are largely those of ethyl alcohol. The acid-form-i bacteria, as stated previously, produce varying amounts of volatile a ds and slight amounts of alcohols and esters. It is likely that the l ger part of the volatile compounds found in the ripening cheese is f med in fermentations which take place subsequent to the initial f mentation of the lactose. The flavor of Cheddar cheese, therefore, o es its origin very probably to the fermentation of the lactose, and to t further change which the products of the initial fermentation un-d go under the influence of biological factors yet unknown. That some

biological factor is concerned in the production of flavor in Chedd cheese is indicated by the fact that if changes are made in the metho of manufacture, changes in flavor are likely to result. If the salt omitted, the typical flavor does not appear. This can be explain only by the action of the salt on certain types of bacteria, which, its absence, are able to grow and produce compounds that are n found in a normal cheese. Apparently the methods of manufactu establish a certain equilibrium in the bacterial life which results in t production of definite substances in amounts varying within certa limits. If any condition is varied too widely, a deviation in the micr bial balance is produced and the products formed in the cheeses a changed in kind or in amounts, either of which may result in a change flavor.

Abnormal Cheeses

The development of a normal texture and flavor in Cheddar chee is largely dependent on the presence of definite types of bacteri If these are replaced, wholly or in part, by other kinds, the product likely to suffer in texture, flavor or both. As has been emphasiz previously, the bacterial content of the milk is of the greatest importan in cheese, since the organisms in the milk pass into the cheese and the produce the same products as they would have done in the uncurdl milk. All abnormalities of the cheese so far as they are occasioned l bacteria are due to the abnormal flora of the milk. To the r material the maker must direct his attention if a fine product is to prepared.

Gassy Cheese.—The most frequent trouble encountered and t one of greatest economic importance is the fermentation caused organisms belonging largely to the *B. coli aerogenes* group. It has be seen that these produce in milk gases, such as carbon dioxide a hydrogen, and offensive smelling and tasting compounds. In chee similar compounds are formed by these organisms; the gas causes t more or less abundant formation of holes which give to the cheese jud an indication of what may be expected with reference to flavor. milk contains some of the gas-forming organisms, but it is only wh they are numerous that marked injury is done.

Gassy cheese may also be due to the presence of lactose-fermenti yeasts which are usually found in milk in such small numbers that th

nnot compete with the lactic bacteria in the fermentation of the sugar the cheese. At times the number may be increased to such an extent at the major part of the sugar is fermented by them, alcohol and carbon dioxide being produced. An outbreak of gassy Swiss cheese was und by Russell and Hastings to be due to such yeasts that had gained trance to the milk from the whey-barrels because of careless washing the milk cans. The cheese makers of the country are realizing the portance of the contamination of the milk from the transportation of hey and milk in the same can. The most practical means of preventg trouble from this practice is to heat the whey to 68° as it passes from e cheese vat to the storage tank. This temperature destroys the rmful microörganisms, and if the storage tank is kept in a sanitary ndition the whey is sweet when returned to the farm in the milk can. has been demonstrated that such a treatment of the whey results in a arked improvement in the quality of the product.

Miscellaneous Abnormalities of Cheese.—*Bitter cheese* is oduced by bacteria that form a bitter principle. An outbreak of tter cheese investigated by Hastings was found to be due to the reacement of the normal acid-forming flora by a lactic organism which oduced such an intense bitterness as to mask the acid taste in the ilk and cheese.

Colored cheese is produced by chromogenic bacteria. In case the olonies are not numerous and the pigment formed is not soluble in any the constituents of the cheese, the color will appear as colored specks, ch as the rusty spot investigated by Connel and Harding, which is due red forms of *B. rudensis*. If the colonies are very numerous, or if e pigment is soluble, the curd may be uniformly colored.

Putrid cheese is caused by the absence of sufficient acidity to hold e putrefactive bacteria in check. This trouble is rare in cheddar eese, since such cheese is made from ripened milk. Fruity flavors are serted to be due to yeasts which form fruit esters.

Moldy Cheese.—In the moist air of the curing-room the cheese forms excellent substratum for the growth of common molds whose pigented spores discolor the surface of the cheese and thus impair its lue because of the appearance rather than by any effect in the flavor. heddar cheese is protected effectively from molds by dipping the eese, when two or three days old, in melted paraffin which excludes e air from the spores on the surface of the cheese.

Specific Kinds of Cheese

There are cheeses made in this, and especially in foreign countries which are of great commercial importance. Only a few can be mentioned. It has been found possible to manufacture a few so-called "foreign cheeses" in this country; however, with some "foreign cheeses" the manufacture has been successful only in such localities where such types originally developed, and where the climate and other conditions are favorable to a normal ripening.

Cheddar Cheese.—*Cheddar cheese*, treated in much detail in the foregoing considerations because it is the most important American cheese, is made in England and her colonies and in the United States

Fig. 137.—Typical development of "eyes" in Swiss cheese. (*Original.*)

It appears in many varieties and by the American consumer is often called American cheese in distinction from the foreign cheeses. This distinction is not wholly applicable at the present time.

Emmenthaler Cheese.—*Swiss or Emmenthaler cheese* originated in Switzerland, but is now made in various other countries. A large amount is made in Wisconsin, Ohio and New York (Fig. 137). It is characterized by its sweetish flavor and by the so-called "eyes," which are holes formed by gas, produced in a fermentation that occurs subsequent to the fermentation of the lactose. The number of eyes is not large and they are evenly distributed throughout the mass of the cheese except near the surface.

The cheese is made from as fresh milk as it is possible to secure. The rennet used is prepared by placing a piece of the dried rennet in whey and incubating the same for twenty-four to thirty-six hours at 30°

his is employed in place of the commercial extract used by the Cheddar aker. It serves not only to curdle the milk, but adds to it a large mber of acid-forming bacteria that have grown in the rennet solution ring the period of incubation. The number is not, however, sufficient to cause any development of acid during the making process which fers from the preparation of Cheddar cheese in the method of firming e curd. This is accomplished by heating the curd to 52° to 60°, and cutting it into pieces scarcely larger than grains of wheat. The salt applied to the exterior of the cheese by immersion in brine for one to ur days and by sprinkling salt on the surface.

The fermentation of the lactose proceeds rapidly during the pressing d subsequent thereto, so that within a few days the sugar has disappared. The lack of the development of acid during the making probably sults in a somewhat different relation between the acid and protein m that existing in a Cheddar cheese, which, together with the abnce of salt gives a somewhat different environment, thus making possible the development of a different flora. There is no ground for believing at the agents concerned in the proteolytic changes are other than those at function in Cheddar cheese. The flavor must, however, be due to her factors; this is indicated by the fact that if the milk is ripened in the Cheddar process, or if salt is added to the curd the flavor will proximate the Cheddar flavor. The formation of the eyes is inhibd by salt, as is indicated by their relative scarcity in the outer layers the cheese. Jensen has shown that the eyes are due to the fermentan of lactates with the formation of propionic and acetic acids, and rbon dioxide. The causal organism is found in the milk and the whey nnet. It is believed that lactic bacteria of the *Bact. bulgaricum* group e important factors in the ripening of Swiss cheese. They are prent in large numbers in the rennet and cheese. Mixed cultures of an ganism of this group and a mycoderma are used with success for the oculation of the whey in which the rennet is to be soaked. The exact le of this form of lactic organism is not known; de Freudenreich conlered them to be concerned in the proteolysis of the paracasein, nce he had found that the content of sterile milk in soluble nitrogen creased when inoculated with the organism. It is probable that e formation of eyes and the flavoring compounds are due, in part at ast, to the same factors.

In the other kinds of cheeses to be described, the rôle of the acid-

forming bacteria is similar, if not identical, to their rôle in Chedda cheese, *i.e.*, in activating the pepsin of the rennet and in preventin the growth of putrefactive bacteria. The factors concerned in flavo development are different.

ROQUEFORT CHEESE.—This cheese, which is prepared almos exclusively in the Department of Aveyron in southern France, is mad from sheep's milk. Its most striking characteristic is the marble or mottled appearance of the interior, due to the growth of a mol *Penicillium roqueforti*, Thom. The curd is inoculated with the mol when it is placed in the press, by sprinkling the curd with bread crumb on which the mold has grown. The growth and sporulation of th mold in the interior of the cheese are favored by piercing it wit small needles, thus admitting air. The characteristic flavor is du partially at least, to the mold.

This cheese is cured in caves having a temperature below 15 The fermentative processes are apparently closely dependent on th moisture and temperature conditions of the curing room. Th emphasizes the importance of biological factors in the ripening proces

GORGONZOLA CHEESE, prepared in Italy from cow's milk, an STILTON CHEESE, made in England are similar to Roquefort i appearance and contain the same mold—*Penicillium roqueforti.*

CAMEMBERT CHEESE.—The soft cheeses are best represented b this important French cheese made from cow's milk by the additio of rennet. The milk is ripened to an acidity of 0.20 to 0.25 per cer before the addition of the rennet. The curd, which thus contair many acid-forming bacteria, is neither cut nor heated in order to r tain the maximum amount of whey. The curd is placed in sma hoops and allowed to drain without pressure. Salt is applied to th surface of the cheese.

The milk sugar is rapidly fermented and the resulting acidit is high, for the cheese contains 60 to 70 per cent of moisture whe fresh and 50 per cent when ready for consumption. The high moistu content of the cheese and the humidity and temperature conditio of the curing room favor the rapid development of microörganism on the surface of the cheese. Both molds and bacteria thrive und the influence of these favorable conditions, changing the cheese t a soft, smooth and butter-like mass, while a characteristic flavor developed.

In three or four days the cheese becomes covered with the growth of *Oidium lactis;* the characteristic mold of Camembert cheese, *Penicillium camemberti,* appears later, within five to six days. These molds reduce the acidity of the curd, and through the enzymes, which they produce and which gradually diffuse into the cheese, proteolyze the curd very completely. The appearance of the cheese when cut indicates the depth to which the enzymes have penetrated; when the entire mass is acted upon, the cheese is ready for use. The reduction of the acidity by the molds exposes the cheese to the attacks of putrefactive bacteria and it soon becomes unfit for use after it is completely ripened. A number of different kinds of bacteria are found in the slimy surface layer, but their rôle is not known.

The development of the characteristic flavor and aroma is dependent on a certain relation between the various biological agents concerned in the ripening. This balance is dependent on very narrow conditions of temperature and humidity; slight changes in these environmental conditions favor or retard the individual types in varying degrees. If the equilibrium essential for the development of typical flavor is destroyed this cheese fails to ripen properly and is of low value. The manufacture of Camembert cheese is a delicate problem in the ecology of microörganisms, and because of this fact the manufacture is attended with greater difficulties than is the case with most types of hard cheese.

CHAPTER IV*

RELATION OF MICROORGANISMS TO SOME SPECIAL DAIRY PRODUCTS

General

There is a number of special dairy products which do not normall come into a discussion of market milk, butter or cheese, but which a of considerable importance. A book of this sort would not be comple without a discussion of some of these products from the bacteriologic standpoint. Some of these special products have been develope as commercial enterprises and the processes of manufacture have be zealously guarded as trade secrets. The result is that there is very litt available data on the manufacture of these products and very litt authoritative knowledge about their bacteriological condition. It therefore, difficult to give a full discussion of the microbiology of the products. A few of the more important ones will be discussed, howeve

Condensed Milk

There are at least three quite distinct kinds of condensed mi made under conditions which result in an entirely different bacteriolo ical condition in the finished product. These different products mu therefore, be considered separately. Condensed milk means simp milk from which a large part of the water has been removed, th decreasing its bulk, the purpose being to lessen the cost of transportati and to increase the keeping quality of the product. Water is remov from milk by some process of heating, either with or without vacuu the heating process being more or less equivalent to pasteurizatic

Sweetened Condensed Milk.—This product is made by reduci cow's milk at the ratio two and one-half to two and three-fourt parts of fresh milk to one part condensed milk, by means of h and the addition of cane sugar. It is then put up in sealed ca

* Prepared by W. A. Stocking.

is not intended to be sterile. The degree of heat to which it subjected is not sufficient to kill all of the microörganisms esent and it is also subject to infection after the condensing completed. Cane sugar is added to the milk, making the final oduct contain about 25 per cent of water, 35 per cent milk solids d 40 per cent cane sugar. The low percentage of moisture together th the added sugar tends to preserve this product against the action microörganisms. There may be some bacterial growth, the idity depending upon the temperature at which the product is pt, but it is usually slow and milk prepared in this way will keep a considerable time without undergoing marked bacterial changes. me gas producing bacteria exist in the milk and if cans containing e organisms are allowed to remain at warm temperatures, they will velop in spite of the large percentage of sugar, producing suffint amounts of gas to cause the ends of the cans to bulge out. Such ns are known commercially as "swell-heads."

Unsweetened Condensed or Evaporated Milk.—In this form of ndensed milk approximately the same amount of moisture is removed in the sweetened product but no sugar is added. The decreased mount of moisture tends to prevent the rapid growth of bacteria, but s is not enough to guarantee the keeping quality of the product. ter the milk is condensed it is put into the can, hermetically sealed, d then placed in steam sterilizers and subjected to temperatures somewhat above the boiling-point. In this way the milk is heated a suffint length of time to insure perfect sterilization of the contents of the ns. If this process is properly done, the finished product contains no ing microörganisms and from the bacteriological standpoint the milk ould keep indefinitely.

Sometimes the unsweetened product is sold in bulk in cans. In s case it is subject to more or less contamination after heating and is t sterile, but because of the small amount of moisture and the concention of the milk solids, the bacteria do not develop rapidly and if pt at a cool temperature, the milk will keep several days without dergoing appreciable biological fermentations.

Concentrated Milk.—There is now on the market a form of conised milk prepared by a different process, which is commonly known as concentrated milk. By this method the water in the milk is removed by means of dry air instead of by vacuum as is the case of condensed

milk. The milk is first heated and then air under pressure is forced through it. By this process the milk is heated to a temperature of 60° (140°F.), and this temperature maintained for two hours, during which time air is forced through the milk causing violent agitation and the removal of the moisture. At the end of this time the milk is reduced to one-fourth its original volume.* The result of this process is a pasteurized milk, with a marked reduction of the original germ content. Investigations by Conn failed to show the presence of *B. coli* in milk prepared by this process. The reduction in the bacterial content of the milk is similar to that secured by other methods of pasteurization. No additional sugar is added to this milk so the product is, therefore, a pasteurized milk containing a small amount of moisture. Because of the small amount of moisture and the concentration of the milk sugar, the bacteria which survive the heating process do not grow rapidly at low temperatures. The following figures will serve to illustrate the effect of this process upon the bacterial content of milk:

Number of bacteria per c.c. in original milk	Number of bacteria per c.c. in finished product
1,250,000	15,000
3,000,000	21,000
518,000	26,000
894,000	9,950
796,000	10,000
150,000	5,000

The rate at which the bacteria develop in this milk is shown by the following counts:

Number of sample	Number of bacteria per c.c.		
	2 days old	4 days old	6 days old
1	18,000	39,000	46,000
2	55,000	28,000	39,000
3	3,500	11,000	10,000
4	4,400	5,270	4,630

The lack of moisture and concentration of milk sugar prevents the rapid growth of these organisms so that bacterial changes do not tak

* Data furnished by H. W. Conn.

place as rapidly as in ordinarily pasteurized milk retaining its normal moisture.

Powdered Milk.—This product is produced by carrying the extraction of the water farther than in the case of the condensed milks. The water is removed to a point where the milk solids can be reduced to a powdered form. This product contains the original milk solids with a very small percentage of moisture usually not more than 2½ per cent. There are several forms of powdered milk now on the market produced by somewhat different methods. In some cases the moisture is removed from the milk by its being exposed to a heated surface in a thin layer. Sometimes the drying is done in vacuum. The resulting product is dry and can be ground to the form of flour.

Another process is to remove the moisture by spraying the milk by means of an atomizer into the top of a hot chamber, the moisture being removed while the fine particles of milk are falling to the floor. By this process the product accumulates on the floor as a very dry flour and does not require any grinding. In the first process the heat is sufficient to pasteurize the milk while in the latter process it is pasteurized before being subjected to the drying process. The powdered milks do not claim to be sterile but are preserved against subsequent action of microörganisms because of the very low percentage of moisture which they contain. It is probable that there is no appreciable increase in the number of bacteria in milk flour and the product will keep for a long time without undergoing bacterial fermentations.

Canned Butter and Cheese

Some effort has been made to put up butter and cheese in hermetically sealed cans, the purpose being to increase the keeping qualities of the products and influence the flavor by controlling the development of the aerobic bacteria. Only a limited amount of bacteriological work has been done on these canned products and the biological changes which take place in them are not very well known.

Special Milk Drinks Made by the Action of Microörganisms

From time immemorial fermented or sour milk has been used as an article of food. We are told that Abraham* placed "curdled milk"

* Genesis 18:8. The Hebrew word "hemah" translated in the English authorized version of the Bible "butter" means "curdled milk." Century Bible, Vol. Judges and Ruth, p. 72.

before his guests and that Moses told the Israelites that curdled m
was one of the blessings which Jehovah had given to his chosen peopl
History also tells us that the wandering tribes of Arabia used fermen
milk as a beverage. For centuries many of the tribes of eastern Eur
and western and middle Asia and parts of Africa have used sour m
for food. Each of these regions appears to have had its own particu
milk beverage resulting from the particular bacterial flora of the regi

The sour milk products which are now on the market unde
variety of names have been derived from these original sour-m
drinks of antiquity. Fermented milk beverages have become v
popular during the last few years among all the civilized peopl
partly because they make a pleasant drink but more especially beca
of their supposed therapeutic value.†

KUMYSS (KOUMISS, KUMISS, ETC.).—Kumyss derives its name fr
the Kumanes, a Russian tribe which lived along the river Kur
This drink was prepared from mare's milk by placing it in a leatl
bag and adding a small amount of old kumyss as a starter.‡ In t
country kumyss is made from cow's milk. This product is now pla
upon the market by a number of companies who keep their metho
so far as possible, from their rivals by maintaining strict secrecy
regard to the methods of preparation. Dr. Piffard§ who has d
special work on this product states that kumyss is fermented by t
action of yeasts and lactic bacteria. This fermentation produ
approximately 1 per cent of alcohol and about 0.75 per cent of ac
Kumyss is strongly effervescent. The lactic organisms used in t
preparation of this material appear to be a strain of the common *Bc*
lactis acidi. Whether or not the yeasts are the common forms u
by bakers cannot be stated with certainty.

Kumyss can be easily prepared in the household by the additi
of cane sugar and baker's yeast to fresh, warm milk which should
kept at a temperature of about 38° (100°F.) until gas begins to for
It should then be bottled and be kept at a cool temperature. In o
or two days a slight amount of alcohol will be formed and a sufficie
amount of carbon dioxide to cause marked effervescence.

* Deut. 32:14.
† Metchnikoff's Prolongation of Life.
‡ Milch Zeitung, September, 1889.
§ New York Medical Journal, January 4, 1908.

KEFIR (KEFYR, KEPHIR, KEFR, ETC.).—Kefir was originally made and used by the inhabitants of the Caucasus Mountains. It was made from the milk of goats, sheep or cows and was fermented by the addition of "kefir grains" to the milk. The origin of these kefir grains is unknown but the natives believe that they were the gift of Mahomet and are carefully preserved by them.

Kefir was prepared by the natives by placing milk in a goat-skin bag and shaking it at intervals until it began to ferment. The kefir grains were then removed, dried and preserved for future use. The fermented kefir was also used as a starter for inoculating new lots. This beverage is now commonly made by more scientific methods.* The principal points to be observed in the preparation of kefir are cleanliness and proper temperature for fermentation and the regulation

FIG. 138.—A large-sized kefir grain and the three species of bacteria of which it is composed. (*From Conn, after de Freudenreich.*)

of the fermentation so that not the acid but the alcoholic fermentation will prevail.† Good kefir should be highly effervescent, should be free from lumps and contain about 1 per cent. of acid but show no marked tendency to whey off. According to Kern, kefir is fermented by a mixed culture of yeasts and bacteria in symbiosis. He found but one form of bacteria present in the cultures he studied. De Freudenreich‡ made an extended study of the flora of kefir. He prepared the kefir from the kefir grains and isolated the organisms present, putting these organisms together in different combinations in order

* Milch Zeitung, 1885, p. 209.
† F. Stohman, Milch and Molkerei Products, p. 1006 to 1013.
‡ Centr. für Bakt. Abt. 2, Vol. 3, 1897.

to determine which were necessary for the proper fermentation the kefir. He found the kefir contained four different organisn yeasts, streptococci, micrococci, and bacilli. The yeasts and strep cocci were plated in gelatin without difficulty but it was very diffic to grow the other two organisms present on any artificial med He concluded that the yeasts present in kefir are not identical wi the species commonly used in making beer and named it *Sacch omyces kefir*. The streptococcus curdled milk in less than forty-eig hours at a temperature of 37° but the micrococcus did not cur milk at all, although it produced a considerable amount of acid.

De Freudenreich changed the name of the bacillus from *Dispora caucasi* given it by Kern to *B. caucasicus*, because it did not produce spores as Kern su posed. He also found that this organism would not grow at all on media with sugar, very slightly on milk, serum, agar, and best of all in milk, in which it produ both gas and acid without curdling the milk. This organism is 5μ or 6μ in leng by 1μ in width, is slightly motile and retains Gram's stain. It has a thermal dea point of 55° for five minutes.

The preparation of good kefir seems to depend upon the combin action of the four types of organisms described. Kefir is sometim prepared without the use of the kefir grains* by placing milk in bottl to which is added a small amount of compressed yeast and sucros The bottles are then held at a temperature of 10° to 15° about fifte hours and shaken occasionally. Kefir prepared in this way gives effervescent mild flavored drink.

LEBEN.—For centuries the Egyptians have used a fermented mi drink known as *leben* or *leben raib*. This was prepared from the mi of cows, buffaloes, and goats. In general it resembles the other fe mented milk drinks in the fact that the fermentation is produc by yeasts and a variety of other microörganisms working togethe At least one yeast and three species of bacteria seem to be norm to this product. A fermented milk drink very similar to leben also used in Algeria. Just the action of each microörganism concern in the fermentation of this product is not certain, but it is probab that all of the species are essential for the production of the particul flavor and consistency of the fermented product. It is claimed th the fermentation that takes place in the milk renders it more diges ible than raw milk. For this reason it is recommended for the u of invalids and persons having weak digestion.

* Milch Zeitung, 1888, p. 393.

YAHOURTH OR MATZOON (YOGURT, YAHOURD, MADZOON, ETC.).—A fermented milk drink known by one of the above names has been used by the Bulgarian tribes for a long time. It has recently been studied and brought to public notice by the investigations and writings of Metchnikoff,* who was struck by the longevity of the tribes using this product as a part of their regular diet. As a result of his investigations, Metchnikoff has advanced his theory regarding the antiseptic power of certain strains of lactic bacteria in the digestive tract. His theory is that certain species or types of bacteria which are able to resist the action of the stomach and can, therefore, pass through into the intestines have the power of checking the growth of the putrefactive bacteria existing there and thereby prevent the production and absorption of bacterial toxins which cause autointoxication. As a result of his experiments, Metchnikoff came to the conclusion that the acid organism (*Bact. bulgaricum*)† found in yahourth was able to establish itself in the intestinal tract and produce enough lactic acid to hold in check the putrefactive processes which otherwise exist there.

Yahourth is made by the Bulgarians in skin bags in the same way the Russian tribes prepare kumyss. It is similar to the other fermented drinks already described in the fact that it is produced by a mixed flora of microörganisms. At least one yeast is present and two or more species of bacilli capable of producing lactic acid in relatively large amounts. These two organisms are known as *Bact. bulgaricum* and *Bacillus paralacticus*. Herter states that *Bact. bulgaricum* is 4μ to 6μ in length by 1μ in width and grows singly or in pairs and occasionally in chains. It stains with ordinary aniline dyes and by Gram's method. It grows with difficulty on ordinary laboratory media and is therefore hard to obtain in pure cultures. These organisms produce a much higher percentage of acid than the common *Bact. lactis acidi* and also grow at a much higher temperature.

This makes it possible to secure it in practically pure cultures by growing it in milk at a high temperature. Grown in pure cultures, the *Bact. bulgaricum* will produce from 1 to 2 or more per cent of acidity. It grows well at temperatures between 37° and 40° and even higher. Recently a number of fermented milk drinks have been put upon the market which have evidently been derived from the yahourth. These

* El., Metchnikoff, Prolongation of Life.

† Hastings has found this organism also common in cow's milk in this country.

are sold under such trade names as *zoolak, vitalac, yogurt, fermenlact* etc. The flora of these preparations appears to be practically the sa as that of the original yahourth.

All of the fermented milk drinks thus far discussed are similar in th each contains a variety of microörganisms, made up of at least o species of yeast with one or more species of bacteria, capable of prod ing greater or less amounts of acid. In some, as in the case of kefir, t yeast fermentation is allowed to predominate, while in others, like y hourth, the action of the yeasts is held in check by the rapid develc ment of the acid by the *Bact. bulgaricum*. All of these drinks are co monly recommended by physicians because of their beneficial eff upon the digestive tract.

Artificial Buttermilk.—In recent years there has developed important industry in the manufacture of artificial buttermilk. T is usually made by inoculating skim-milk with a culture of lac bacteria, either *Bact. lactis acidi*, or *Bact. bulgaricum* or a combinati of these two types. In making the artificial buttermilk, yeasts are r commonly added. After the milk becomes coagulated, it is th churned in order to give it a smooth, creamy consistency, after wh it may be bottled and kept for some time by holding at low tempe tures. Sometimes a small percentage of whole milk is added at the ti of churning to make the finished product more closely resemble natu buttermilk. In making artificial buttermilk, the skim-milk is f quently pasteurized in order to get rid of the miscellaneous flora wh it contains. The finished product, therefore, differs from ordinary b termilk in the fact that it contains nearly pure cultures of the lac organisms while the natural buttermilk will contain a more or l miscellaneous flora in which the acid organisms predominate. It possible to obtain a more uniform product in the artificial butterm than in the natural product, and this is perhaps responsible for t rapid development of this industry. All of these fermented m drinks contain enormous numbers of microörganisms, usually millio per c.c.

Frozen Milk

Some effort has been made to put upon the market milk which l been frozen into cakes or bricks. This has been tried both in Eur and in this country. Some difficulty has been met in satisfactorily fre

ing he milk and holding it in a frozen condition. The process has pro ed to be rather expensive and not very satisfactory. One difficulty wit this process seems to be that the quality of the frozen milk after it has een melted is not as good as it was before it was frozen. From a bac riological standpoint, this process is of some interest, but it is dou tful whether it becomes of much importance commercially.

Ice Cream

ce cream is one of the important manufactured dairy products and its se seems to be increasing steadily. Its bacterial flora varies with the aterials used in its manufacture and the conditions under which it is ade. It may be made from fresh cream which is only a few hours old nd under good sanitary conditions. On the other hand, it may be ma from cream which has been produced and handled under unsanitar conditions, kept in storage for a number of days and finally manufac red in surroundings not conducive to a low bacterial content. We are ot surprised, therefore, to find a very wide variation in the germ con nt of ice cream, as it is placed upon the market.

n examination of 263 samples of ice cream collected in the city of Wa ington* showed an average germ content of over 26,600,000 per c.c. The lowest count obtained was 37,500 and the maximum was 36 ,000,000. A similar study of commercial ice cream in Philadelphia† sho ed the average bacterial content to be very high. The lowest cou t found was 50,000 per c.c., while the highest count was 150,200,000. In is work it was found that the bacterial content of the ice cream was in ite direct relation to the sanitary conditions of the establishment whe e the ice cream was manufactured. When ice cream is manufactur l in a city from materials which have been shipped in from consider- abl distances and frequently held for several days in cold storage, it is not surprising that the germ content of the manufactured product sho ld be high. In some establishments the cream is pasteurized before manufacturing, while in others it is used in its raw condition.

n normal cream held for sometime, the lactic bacteria should exist in nsiderable numbers, but when cream is held at low temperatures the organisms do not develop rapidly. Pennington found that cer-

*Results of work done under the direction of G. W. Stiles.
†Work done under the direction of Dr. M. E. Pennington.

tain species of streptococci developed quite rapidly in cream held t refrigerator temperatures. Streptococci were found in fifty-five (80 r cent) of the sixty-eight samples examined. It was found that at refi- erator temperatures the relative growth of these organisms was grea r than at higher temperatures, a fact which may account, in part at le for the frequency with which these organisms occur in ice cream.

Frequently ice cream is held for a considerable time in a frozen c- dition before it is sold. It has generally been supposed that there is bacterial growth in material which is held below the freezing tempe- ture. This, however, did not seem to be the case in samples exami i by the investigators already mentioned. They found in samples h i about a month that there was normally a decrease in the bacterial co t and also in the amount of gas production for a number of days, a r which there was frequently a marked increase in the bacterial cou . These results would seem to indicate that even in the frozen condit n there may be some increase in the number of bacteria present. e number of these experiments, however, is not sufficient to justify v y general conclusions. The work of Conn and Esten* in holding milk t 1° may throw some light upon this question.

If the cream from which the ice cream is made has been produ d and handled under sanitary conditions, the bacterial content sho d consist chiefly of organisms of the *Bact. lactis acidi* type, in which c e the high count in the ice cream might not be objectionable. If, n the other hand, the cream has been held in cold storage for some t e under conditions which inhibit the growth of the lactic organisms a d permit the development of putrefactive types, bacterial poisons n y be developed in the cream, which will be highly objectionable. Th e seems to be little doubt that this is the cause of the cases of ptom n poisoning, resulting from the use of ice cream. It is known that cert n types of bacteria, especially those belonging to the so-called pu - factive group, are capable of developing at very low temperatures d can, therefore, produce considerable quantities of toxic products n the cream. Whether or not these products are developed bef e the cream is manufactured or whether they may develop in the fro n product cannot at present be stated. In general it can be said that e total bacterial count does not indicate the wholesomeness of the e cream any more than does a similar count in buttermilk or in the c -

* Annual Report, Storr's Experiment Station, 1901.

mercial fermented-milk drinks. The kinds of organisms present is a far more important question from the standpoint of the wholesomeness of the ice cream. However, the results obtained by many ice-cream manufacturers has demonstrated the fact that the germ content of this product can be quite definitely controlled by the same methods of care and sanitation as are required in the handling of other forms of dairy products.

DIVISION V

MICROBIOLOGY OF SPECIAL INDUSTRIES

CHAPTER I*

DESICCATION, EVAPORATION, AND DRYING OF FOOD

FACTORS THAT BRING ABOUT CHANGES IN DRIED FOODS

The factors that bring about changes in dried foods may be consider under two general heads, chemical and microbial. Enzymes, althou the product of living cells, may represent the chemical changes, and t activity of bacteria, yeasts and molds, the microbial changes. Enzyn are normally present in food stuffs derived from animals or plants wh have not been subjected to heating. All living cells contain enzym and these may remain active for a considerable time after the de of the cell. Some of these enzymes attack carbohydrates, some fa some proteins, and some other compounds. Enzymes are responsi for the stiffening of the muscles after death (*rigor mortis*), others la break down the tissues and bring about a ripening of meat whereby becomes more tender. Autolytic enzymes may in some instan produce rancidity in food products by a splitting of the fats. Bacte enzymes are known that duplicate the action of practically all th produced by higher animals and plants. Some of the changes p duced are desirable, others undesirable, particularly if action is allow to continue for too long a time. In foods dried for preservation, i therefore important that sufficient heat be used to destroy the enzyn or that enough water be removed to inhibit their activity. Ordinar the activity of enzymes will be inhibited by the removal of wa sufficient to prevent the growth of microörganisms. The action enzymes is characterized as reversible, that is, after a certain conc tration of enzymic products has been reached, the transformation cea

* Prepared by R. E. Buchanan.

til a part of the accumulation has been removed by diffusion or otherse. Since many of these actions are hydrolytic in nature, water for h diffusion and hydrolysis must be present before the enzyme n act.

Bacteria are introduced in large numbers when food is handled and bably constitute the most important factor in its destruction. If isture and temperature conditions are favorable, they bring about desirable changes. The amount of water present in foods may be d as a basis for their classification into four groups: first, those in ich moisture is present in appreciable quantities in the interstices, t is, those which seem *wet*. Under these conditions bacteria not ly multiply but spread rapidly through the medium by actual space wth, by diffusion currents, and by their own motion. Second, ne foods may contain sufficient moisture for the abundant growth of cteria, but not free water for diffusion and distribution. In these e spread of infection must be largely by direct growth of the organism d will necessarily be slower than in the preceding. Third, the subatum may be so dry that little or no growth of organisms may take ce, yet there is sufficient moisture so that they remain viable. urth, the food may be so dry that only those organisms that can thstand relatively complete desiccation will survive. These groups not be differentiated entirely upon the basis of the percentage of ter present, for the character of the food itself and of the material solution are also important.

Yeasts usually require sugars for their best development and are erefore commonly present in foods containing this substance. They e of importance therefore in fewer foods than bacteria. In nature, ey are frequently found upon fruits, particularly those which contain nsiderable quantities of sugar in the sap. They will be found also on the cut ends of twigs or grass culms where sugary sap has oozed t. Colonies of considerable size may sometimes be seen upon corn bble during damp weather. They are commonly distributed by s and other insects which feed upon the sugary plant juices. They e not motile, hence the spread of infection in any food must be by rect growth.

Molds, like bacteria, are ubiquitous and under proper conditions ll destroy almost any food. They grow readily in solutions and on turated substrata, but ordinarily are prevented by the bacteria which

find the optimum condition for their growth in such conditions. Fo example, it is commonly observed that wet silage rots when expose to air supports a luxuriant growth of bacteria, while drier silage become moldy. Unlike bacteria, the molds extend through and over food whe there is no visible water film. The spores are much better adapted t air dispersal than are bacterial cells, and the hyphæ penetrate mor rapidly than will the bacterial colony. In certain foods, therefore, a meals and flours, molds are more destructive than are the bacteria Usually they will multiply with less moisture.

Inhibition of Growth of Microörganisms in Dried Food

In a few cases, the development of microörganisms is prevented b the absence of sufficient moisture in the medium to support growth This is not nearly so common as might appear at first thought. I occurs in some foods as olive oil, starches, meals, cane sugar, etc., tha have little or no free water. Frequently the drying results in a cor centration of the solutes, beyond the point to which microörganism can adapt themselves to the osmotic pressure. When it is remembere that a 50 per cent solution of cane sugar is capable of exerting a pressur of about 226 kg. (500 pounds) per square inch, it will be realize that considerable readjustment is necessary in the cell of a yeast plan that can grow in such a medium. Drying also sometimes changes th former relationship of cells and tissue constituents so that protectiv layers may be formed. For example, in curing pork, the fat which i structurally isolated in distinct cells for the most part becomes diffuse throughout the outer layers of the tissues and forms a water-free an water-proof exterior. Foods are sometimes subjected during th process of drying to sufficient heat to destroy the microörganisms cor tained. At other times they are exposed to the germicidal action c the direct rays of the sun or to the fumes of some disinfectant c bleaching agent as sulphur dioxid or smoke.

Methods of Drying

The reduction of the water in foods below the minimum require for the growth of microörganisms is accomplished in a variety of ways Most commonly heat is employed, either the sun's ray or some artificia

urce. In localities where the humidity of the air is low, as in many the irrigated fruit districts of the western United States, exposure the rays of the sun results in rapid drying. With other types of ods and in more humid regions, artificial heat is used to reduce this lative humidity. Some foods cannot be dried at high temperatures cause of their instability. In most cases such foods must be dried ickly for they are readily attacked by microörganisms. These are ually dried at a low temperature and in a partial vacuum. Other ods are dried without recourse to evaporation by the use of the ydraulic press or by centrifugal action, the latter in the manufacture cane sugar. The water available for the growth of microörganisms ay be reduced by the addition of some crystalline substance such as gar or salt. The usefulness of the latter depends largely upon their bility to create a concentration of solutes too great for the growth of acteria. At the same time a considerable proportion of the water om that part of the food into which the solutes will not penetrate, is bstracted by osmosis.

Many food products do not require any additional drying, as they aturally contain little moisture. Such are the grains and the products anufactured from them, as flour. The drying in this instance has curred during the ripening process of the grain. When for any ason this does not occur, the grain will mold. It has been found ecessary in many instances to kiln-dry corn. Grain, nuts, etc., are by eir nature adapted to keep under normal conditions for considerable eriods. Other foods require artificial drying. In these we have the ntergrading classes, which have been discussed above, those which ntain a very small percentage of water and those which have con- derable water but a high concentration of solutes. The absolute mount of water in the food is by no means an index to the amount at is available for the growth of microörganisms. Many foods are ygroscopic. Foods having the same water content and percentage f solutes will behave very differently with reference to delivering up e water to any organism present.

The effect of the concentration of solutes by drying is perhaps the ost important factor in the preservation of food. These substances issolved in the water may be actually antiseptic when concentrated, s the acids of the juices of certain fruits. More often the sugars each a concentration so great as to prevent growth by plasmolyzing

the cell contents of the organisms. For every organism there is a maximum concentration reached sooner or later, beyond which growth is impossible.

Dried foods may be divided into three groups, using the relative abundance of *carbohydrates*, *fats*, and *proteins* as a basis for classification.

Carbohydrate foods are usually preserved by drying. Many, such as grains and nuts and the flours and meals prepared from them, do not require artificial heating. They are, however, somewhat hygroscopic and in damp climates enough moisture is taken up to allow the growth of injurious molds and bacteria. Moldy corn has long been regarded as the probable cause of the disease, called pellagra, in man. Still other carbohydrate food stuffs require more or less care in the drying or curing, such as hay and fodder in general. This is usually dried by simple exposure to the air and sun until most of the water has been evaporated. Fodder that has become moldy through the presence of too much moisture is a prolific cause of trouble in horses and less frequently in cattle. The many deaths due to the so-called cerebrospinal meningitis are suspected many times to be due to the consumption of moldy hay. In localities where the air is too moist or it rains so frequently as to make it difficult to dry hay, curing is effected by a process of self fermentation. The hay is piled in a mass while still green and undergoes a process of heating. The temperature rises usually to about 70°. The causes of this rise are somewhat uncertain, but it is probably due to the combined action of enzymes and microörganisms. Just how much of this keeping quality is due to the heating, how much to the loss of water, and how much to the accumulation of products of fermentation is uncertain. In other cases, the heated hay is spread out and quickly dries sufficiently so that it may be stored. A certain small percentage of the nutriment of the hay is necessarily lost in the development of the heat energy.

Fruits are quite generally preserved by drying. In many instances, as in peaches, apples, and berries, it is probable that enough moisture is usually removed to prevent organisms from growing, but in many other cases, as in the preparation of currants and raisins, the concentration of the sugar and other solutes is the controlling factor. Frequently as much as 30 per cent of the dried fruits is water. Fruit drying is often accomplished by the heat of the sun's rays, in other cases artificial heat or even hydraulic presses are used.

Many manufactured products, particularly baker's goods, as crackers, biscuits, dried yeast cakes, etc., are preserved by the elimination of water.

Macaroni and vermicelli are prepared by forcing a thick paste of especially prepared flour and water through openings of different sizes. The product is then dried in the air until it is brittle and may then be kept indefinitely.

Copra, one of the principal exports of certain of the islands of the Pacific and Indian Oceans, is prepared by cutting the meat of the cocoanut into pieces and drying them in the sun. From this copra, much of our desiccated and powdered cocoanut is prepared.

Syrups, molasses, jellies, jams, and many other carbohydrate foods are preserved through the concentration of the solutes. Many of these are partially sterilized by the heat used in the process of manufacture, but there is usually plenty of opportunity for subsequent infection. They are more frequently attacked by molds and yeasts than by bacteria. An exception may be noted in *Streptococcus mesenterioides* which sometimes causes considerable trouble by a gelatinous fermentation in syrups from which sugars are manufactured commercially.

Foods with considerable quantities of *fat* usually contain little water. Cottonseed, olive and other vegetable oils, the plant and animal fats, as lard, tallow, and butter, are quite resistant to change by bacteria unless water is present and considerable traces of nitrogenous impurities remain in them. With these foods the water is necessary for the growth of the organism and also for the activity of the lipolytic enzymes, which might hydrolyze fats and aid in the development of rancidity. Butter forms an exception to the rule that fat foods contain little water, as it usually has from 12 to 16 per cent. Where it is necessary to keep butter-fat for long periods or under unfavorable conditions, it is melted, the water and the nitrogenous impurities removed, and the clear fat preserved. Bacteria, enzymes, and a few molds have been described that attack fats. In the process of preparation or manufacture of any fat foods, sufficient heat is used to sterilize the material and infection thereafter spreads to the interior very slowly. The heat destroys the enzymes as well as the bacteria.

The third class of *foods* preserved by drying includes those that contain a high percentage of *protein*, in large part flesh foods and flesh derivatives.

Desiccation, however, is only one of the agencies acting to preserv the flesh.

Jerked meat is sometimes prepared in localities with a hot dry climate. Lea meat is cut into thin slices and exposed to the direct rays of the sun until dry. Th bactericidal action of the sunlight and the rapid extraction of moisture preven microörganisms from producing undesirable changes during the curing process.

Dried beef is lean meat which usually has been treated with certain condiments smoked and salted and then dried.

Dried fish such as cod, mackerel, and herring, is prepared by the use of cond ments, salt, and smoke in addition to the drying.

Pemmican is prepared by drying lean meat, grinding it, and mixing it with sug and fat, dried fruits, spices, etc. It is highly nutritious, not unpalatable, and cor pact, and will keep for a long period. It is frequently used as a concentrated for of food by Arctic explorers, etc.

Beef extract is prepared by cooking minced beef and water in a receptacle und a slight steam pressure. The digestion is continued for several hours. The liquid filtered off and concentrated in a partial vacuum to the desired consistency.

Gelatin is prepared by boiling bones and tendons, sometimes also horn and hi scraps and concentrating the gelatin which dissolves from these.

Somatose, sarco-peptone and related so-called predigested protein foods are mi tures of albumoses and peptones prepared by the artificial digestion and drying proteins, usually flesh. The product is marketed as a powder.

Milk, either with or without its butter fat, is dried by being sprayed into a war compartment from which the air is partly exhausted. It dries immediately, in th form of a very fine powder. This powder, if thoroughly dry, will keep well and finding an extensive use. The high sugar content of this powder is instrumental i preventing the development of microörganisms.

Eggs are dried in much the same manner as milk and the product is being use extensively at the present time by bakers.

Meats are frequently preserved by a combination of drying and th action of certain antiseptics or preservatives. The salting of meat owe its effectiveness in part to the abstraction of water. In most case the surface of the meat and probably even the other portions ar protected in large measure by the diffusion of the fat and the satura tion of tissues and by the formation of water-proof fat films. Th autolytic enzymes are active in the fresh meat and soon becom inert upon the removal of water. The organisms responsible fo decay of preserved meats and flesh foods are usually bacteria. Som of these break down the protein into simpler chemical compounds, c which a few are poisonous.

CHAPTER II*

HEAT IN THE PRESERVATION OF FOOD PRODUCTS

Historical Résumé

The principle involved in the preservation of food by heat may be sd to have had its origin in the experiments of Spallanzani, who in 15 boiled meat extract for an hour and hermetically sealed the flasks, aer which treatment no change occurred in the material. An applica-tn of this principle was suggested as early as 1782 by the Swedish cmist, Scheele, who advised the exposure of vinegar in bottles to the tmperature of boiling water in order to effect its preservation. Some yrs later the principle was applied to the conservation of food by a Fnch confectioner, Nicholas Appert, who in 1811 published an eaustive treatise on "The Art of Preserving Animal and Vegetable Sbstances." His method was to enclose the food in a glass jar which ws then corked tightly, and placed in boiling water, the length of tie of heating varying with the article treated.

In 1810 Peter Durand secured a patent from the English governmnt for the preservation of fruits, vegetables and fish in hermetically sled tin and glass cans. He did not claim to be the discoverer of the pcess, but said it had been communicated to him by a "foreigner reding abroad." Although the secret of the process was jealously grded, the employees of different establishments became familiar wh its essentials, and in this manner the industry found its way to Aerica. One of the first to introduce the process was Ezra Daggett, wo, with his son-in-law Thomas Kensett, in 1819 engaged in the manufaure of hermetically sealed goods, the principal foods packed being sanon, lobsters, and oysters. In 1820, William Underwood and Crles Mitchell, emigrant employees from a canning factory in England, oned a factory in Boston where they canned plums, quinces, cranberies and currants.

In the earliest days of canning, glass jars were used exclusively, but

*Prepared by S. F. Edwards.

were gradually abandoned as it was found that they could not readil withstand the extremes of temperature, and were expensive, bulk and costly in transportation. In 1825, Thomas Kensett secured patent on the use of tin cans in preserving food, and in the same ye began using the process in his factory. The early manufacture of t cans was by hand and crude, the bodies being cut with shears and t side seams made with a plumb joint (that is meeting but not ove lapping), and then soldered together. Heads were made to set in the body and were soldered in place in a very crude manner. T making of 100 cans was considered a good day's work for one ma Improvements were gradually made, however, in their manufactu until finally can making became a distinct industry and now all t parts are made and put together by mechanical devices.

In the original Appert process, the goods were cooked in open kettl the highest temperature obtainable by this method being the boili point. A little later common salt was used to aid in securing a higl temperature, and this was followed later by the use of calcium chlori which made possible a temperature of 115°. In 1874, a closed ket was invented for superheating water with steam, and this was imm diately followed by another improved kettle in which dry steam w used, the principle employed being that of the modern autoclav, which method any desired temperature may be obtained and modif to suit the requirements of different classes of food.

Economic Importance

From Standpoint of Health and Dietetics.—The value o variety of foods, especially fruits and vegetables, is recognized dieticians. Unfortunately, however, the season for fresh fruits a vegetables is comparatively short. Moreover, many foods gro exclusively in one section of country will not withstand shipping i fresh condition to other sections. In spite of improved method refrigeration, it is not practicable to ship fresh sea foods to far inl towns, or to send some perishable products of warm climates to c countries. The canning and perserving industry overcomes th difficulties by supplying pure, clean, wholesome fruits, vegeta meats, and fish to any region the year round, and at prices c paratively low.

From Standpoint of Commerce.—In its commercial aspect, the importance of the industry can scarcely be estimated. Canned products make possible the carrying of larger stores of provisions by armies and navies and expeditions for exploration than would otherwise be possible. In fact, the stimulus which prompted the investigation of Appert was a prize offered by the French Navy Department for a method of preserving foods for provisioning ships more satisfactory than by pickling, drying, or other methods in use up to that time.

"Although the preserving industry was established in three great commercial centers in the United States as early as 1825, it did not become of much importance until the last decades of the nineteenth century. There were many hindrances to the progress of the industry, such as the secrecy observed in the process, skepticism of the public regarding the healthfulness of canned foods, the general prejudice against them, and the high cost of production. These obstacles have gradually been surmounted, and at the present time the several branches of the industry have collectively assumed large proportions.

An idea of the magnitude and importance of the industry in the United States may be gained from statistics for 1914 compiled by the National Canners' Association, and here reproduced by permission. The pack of tomatoes was 15,222,000 cases; of corn, 9,789,000 cases; and of peas 8,847,000 cases. This does not include fruits, the pack of which in California alone in 1912 was 4,833,900 cases. Nor do these figures include the great variety of other vegetables, fruits from other states than California, preserves, oysters, meats, or fish. The average case holds two dozen cans, and sells at an approximate average price of $2.40. It is apparent from these data that the canning and preserving industry is one of immense value, and that it constitutes a large factor in the feeding of the race."

Alteration of Food

Physical Changes.—*Appearance.*—Some physical changes attend the conservation of foods by heat, approaching more or less closely the changes incident to the ordinary preparation of fresh foods for the table. In the preserving of some vegetables, notably peas and asparagus, the canner subjects them to a blanching process which consists in submitting the vegetables to the action of hot water for a short

time, the object being, first, to remove the mucous substance from t outside and a part of the green coloring matter so as to have a cl liquor in the can, and second, to drive water into the vegetables so t all will be tender. The blanching process also improves the color these vegetables.

Mechanical Disintegration.—In the case of very soft fruits or ve tables, the high temperature essential for sterilization causes a sli amount of mechanical disintegration, which is not objectionable, h ever, unless excessive, as there is little deterioration in appearance, a none in food value. In the case of meats, practically the only physi change is the shrinkage during the parboiling previous to placing the cans.

Chemical Changes.—*Appearance.*—The chemical changes in fo preserved by heat may be considered under two heads: first, those which the appearance is modified; and second, those in which the f itself is altered. Some change of color sometimes occurs and res from various causes. In colored vegetables, such as peas, string be and asparagus, a part at least of the loss of color is due to the oxidat of chlorophyll. With a few foods, iron sulphides are occasion formed by a combination of sulphur of the foods with the iron of container. This seldom occurs however, and is not of great imp tance. Some fruits packed in glass gradually lose their color by ox tion on exposure to the light.

Chemical Change.—So far as chemical alteration of the food it is concerned, there is little change and none other than would occu the ordinary preparation of the food for the table. The albumins coagulated. The fats probably remain unchanged. Of the ca hydrates, the chief action is on the sugars. The cane sugar is wh or partly inverted by the combined action of the heat and the frui vegetable acids. The starch undergoes little if any cleavage, inasm as this change occurs only in the presence of acids, and in foods wi high acid content, the proportion of starch is relatively low. The o amyloses probably undergo little if any change.

Palatability and Digestibility.—It is often contended that can foods are less palatable than fresh foods of the same kind. This of agreeableness to the taste is, however, more seeming than real, arises largely from the prejudice of the consumer against food conse in tin cans, rather than from any actual change. When the preser

is roperly done the product should be no less attractive to the eye, no lespleasing to the palate, and of no less value from the standpoint of di stibility than the same food when served in the fresh condition.

Biological Changes. *Vital Disorganization.*—The entire industry of onservation of foods by means of heat is based on a microbiological pr ess. It is a universally recognized fact that the ordinary spoilage of fo is a microbiological change, hence the individual desirous of pr ecting food from spoilage must give consideration to the microbial ag ts responsible for the change.

Normal Flora and Fauna.—Aside from a few types of organisms ca ing disease or specific poisonous conditions, we are unable to desig na definite species as those causing canned goods to spoil. Consider in he great variety of foods preserved by heat, and the different con di ns under which they are grown and secured, it naturally follows th the normal flora and fauna of food to be preserved in this manner w ld embrace a wide variety of species, including some higher fungi, m ls, yeasts, bacteria, and low animal forms. Generally speaking, th microbial flora of fruits consists mostly of molds and yeasts, al ough bacterial forms may also be present. In the case of vege ta es, and of fruits coming in contact with the earth, more species of ba eria are apt to be present, many of them spore formers able to wi stand a high temperature. Finally, in meats and fish the living fo ns may include not only molds, yeasts, and bacteria, but animal fo ns as well, such as the organisms of tæniasis (tapeworm) and tri inosis. In the preserving industry, therefore, consideration must be iven to all these forms, not only from the standpoint of the suc ce ul preservation of the various foods, but also from that of pro te ng the health of the consumer from possible poisonous substances pr ent or produced in the conserved food, and from possible infection wi pathogenic organisms present in the food before it is packed.

Pasteurization

Economic Considerations.—In the preservation of food by heat, tw processes are applicable, pasteurization, and sterilization. In pa eurization, the aim is not to effect the permanent preservation of fo s or drinks by destroying all life present, but rather to destroy ce in species of organisms, thus checking the natural fermentation, an effecting a temporary preservation.

The principle of pasteurization may be said to have originated in the early work of Spallanzani and Scheele, already mentioned, and was employed by Appert in his later investigations. The operation as carried out by Appert does not, however, appear to have found general application until Pasteur revived the method, and as a result of his activities in attempting to secure a general adoption of the practice to prevent the spoiling of wine, the process was named from him.

SPECIFIC APPLICATION. *Beer.*—Pasteurization is of economic importance particularly in the dairy and fermentation industries. In brewing, "The process of pasteurization is in use with even the small brewers in the United States, beer being pasteurized even for local consumption." The beer is pasteurized in bottles by being subjected to a temperature of 58° to 63° for one-half hour. The entire process as practised in the large breweries, requires less than an hour, and includes the warming of the cold bottles to pasteurizing temperature, the pasteurizing proper, and the cooling to a little above room temperature. The process is a continuous one, the bottles being put into the machine at one end and taken out at the other. Experiments have been made in pasteurizing beer in large containers, steel, copper, aluminum, and tin having been tried, but without complete success as yet.

Fruit Juices.—The essentials in the pasteurization of wine and fruit juices are similar to those for beer. There is, however, no universal rule of application. Details of the process must be arranged to fit the character of the different liquids under treatment.

Cream and Milk.—Pasteurization as employed in the dairy industry varies in its method of application according to the purpose for which it is used. In factory butter making, it must be employed to secure the best results. Milk as ordinarily received at creameries contains a widely variant microbial flora, many of the species exerting a greater or lesser influence in determining the flavor of the finished product. By pasteurization of the cream, the butter-maker destroys most of the organisms present; and by the use of a culture starter of lactic acid bacteria, he is able to control the fermentation, and is assured of a uniform quality of product from day to day throughout a season. An added value of pasteurization is that all pathogenic organisms are destroyed, thus aiding in the prevention of such diseases as might be

nveyed through this product. In creameries, the usual process of steurization is what is known as the continuous or flash process, in hich the milk is subjected to a momentary heating to about 85°, e flow through the pasteurizing machine being so regulated as to ing all the milk up to the desired temperature, the heating being mediately followed by rapid cooling, and subsequent addition of the ctic starter.

In the pasteurization of milk for infant feeding, a lower temperature employed. A temperature sufficiently high to kill the organism of berculosis (the standard for pasteurization) by momentary heating, parts to the milk a cooked flavor, making it less palatable, and coagules some of the protein constituents making it less digestible. The sired end may be reached however, by using a lower temperature for onger period of time, and the method generally recommended is to at the milk to 60° to 65° for thirty minutes. This heating is sufficnt to render harmless any pathogenic organisms likely to be present the milk, without the objectionable features attendant on heating a higher degree.

Condensed Milk.—It is commonly stated that Gail Borden invented e process for preparing condensed milk, in 1856. Previous to this wever, milk had been condensed in France, Germany, and England early as 1825 to 1835. While he cannot, therefore, be called the ventor of condensed milk, to Borden belongs the credit of having st prepared it by a rational process, and in a practicable form.

In the manufacture of condensed milk, good fresh milk is evaporated a vacuum pan similar to those used in sugar factories, at a temperare of 40° to 50° until the volume is reduced a little more than half, ne sugar being added so that the finished condensed milk usually ntains 40 per cent. cane sugar. The evaporation must be conducted th great care, otherwise the lactose crystallizes out, and this causes e product to feel "sandy" to the tongue. When the evaporation the milk is complete, the yellowish white syrup is sealed up in tins hich hold about 450 g., and this quantity is equivalent to about 1½ l. normal milk. The addition of cane sugar acts as a preservative, d although the finished product may contain some living organisms, is said to keep indefinitely if unopened, and will even keep for a mber of days after opening. Occasional losses do occur by spoilage the finished product, either from the growth of occasional types

of bacteria tolerant of the high percentage of cane sugar, or fro yeasts.

Sterilization

Economic Considerations.—For certain classes of food produc pasteurization is widely applicable, and is of immense value from economic standpoint. Preservation by pasteurization is at best, ho ever, temporary. Spores of spore-forming bacteria are certain to present on many kinds of foods, and these, unharmed by pasteurizi temperatures, develop vegetative cells, and spoilage occurs.

For permanent preservation, therefore, sterilization must be adopt and it is upon the principle of sterilization, coupled with prevention future contamination by hermetically sealing the container, that t whole canning and preserving industry is based. The method applicable to nearly every class of food, and with less alteration in t food than any other method of conservation. The principle employ to-day is essentially the same as that used by Appert over 100 ye ago. Although he knew nothing of microörganisms or their relation the spoilage of food, Appert's experiments taught him that not o must the food to be conserved be heated thoroughly, but that it m be so sealed as not to allow air to enter the container. In the light microbiological science, it is clear that the success of the process depen not upon keeping out the air, but upon keeping out organisms which carried in the air.

Specific Application.—The process of preservation by ste ization is not so extensively practised for fruit juices and ferment liquids as that of pasteurization. If too high temperature is employ for fruit juices, certain compounds of agreeable taste and aroma destroyed, with a consequent deterioration in the flavor of the produ Fruit juices may, however, be sterilized by heating at a low temperat for several successive days.

The method of Appert has its widest application in the conservation of fruits vegetables, meats and fish. Whatever modifications are made in the handling of different classes of foods the essentials are the same. The raw material a thorough cleaning and removal of waste if any, is filled into the cans and submit to the sterilizing process, the degree of heat and time of processing varying v different products. From the character of the flora, fruits as a rule require a c paratively low temperature for sterilization, while some vegetables and meats

que a very high temperature to destroy the bacterial spores sure to be present. Briefly, the methods employed in canning some foods follow:

Meat.—In the meat-canning industry, lean meat is largely selected for two reasons. Fat well-finished carcasses bring a better price when offered for sale in the fresh condition; and in the second place, lean meat has a better appearance in the canned state than fat meat. The selected meat is cut into pieces of approximately from 1 to 4 pounds in weight, according to the size of the tins in which it is to be preserved. The pieces are cut as nearly as practicable the same size, not only for purposes of appearance in the cans when opened, but also that the process of sterilization may be more uniformly carried out. If the pieces were of different sizes, the smaller ones would become thoroughly cooked and disintegrated before the larger ones were sterilized.

After the pieces have been selected and dressed they are parboiled before being packed in the containers, the time ranging from eight to twenty minutes, according to the size of the pieces of meat. The object of parboiling is to secure the shrinkage which always takes place on heating. Meats put into tins in the fresh state and sterilized shrink to about two-thirds of their original volume. When the meat is put directly into boiling water, there is less loss of protein than when the meat is packed in cold water and heated gradually. During parboiling, the meat loses about 1 per cent of the protein content, about one-third of the total meat bases, and 50 per cent of the mineral matter.

This shrinkage by parboiling tends to make a more concentrated article, thus favoring transportation, and, pound for pound, the nutritive value is not lowered. Practically, the nutritive value of a pound of properly canned beef is about one-third greater than that of 1 pound of fresh beef of the same kind. After parboiling, the meat is placed in tins, by hand or by machinery, and to each can is added a small quantity of "soup liquor," the manner of preparation of which is not disclosed by the packers. It may be regarded as a thin soup, the object of which is to fill up the spaces between the meat, and to add condimental substance to render the meat more palatable.

After the cans are filled, they are closed and processed in suitable retorts by steam under pressure, as previously described, the temperature ranging from 110° to 120°. A modification of the usual method consists in exhausting the cans *in vacuo*, and automatically sealing them in the exhausted state, thus removing all the air and other gases, after which they are placed on an endless conveyor and dipped into an oil bath at a temperature of 115°, the speed of the conveyor being so regulated that the cans remain in the bath a sufficient length of time to complete sterilization before they are carried out at the opposite end. They are next carried automatically into a solution of carbonate of soda, and finally into pure water, after which they are dried, painted with a shellac or lacquer and labelled.

Fresh meats other than beef or pork are canned in a fresh state. When game and wild fowl, as well as domesticated chickens, ducks, geese, turkeys, and pigeons are used, the general process is as already described. Horse meat is used more or less commonly in some European countries, but probably rarely in the United States.

Fish.—The process of fish canning does not differ materially from that of other

meats. On account of its proneness to rapid decomposition, especial care must observed that the fish are in a perfectly fresh state before canning, and that sterilization be most thorough. Salmon is the principal fish for the preservation which dependence is placed on sterilization alone, most fish being preserved other methods.

Vegetables and Fruits. Corn.—The young tender ears of sweet corn are pic from the stalk, preferably in the early morning, keeping the husks on, and taken in this condition to the factory. They are husked and the silks removed passed through machines with sets of knives which cut the grains evenly from cob, care being observed not to cut the corn so closely as to cut off particles the cob with the corn. After the corn is cut off the cob, some canners add a "syru of water, salt and sugar, and cook the corn for a few minutes at 80°, after whic is filled into the cans, sealed, and sterilized. Another method is to fill the uncoo corn directly into the cans, fill them with "syrup," hermetically seal and steril The temperature employed varies somewhat, but usually lies between 115° 121° for thirty minutes for No. 2 cans holding 20 ounces. Proportionately lon time is required for larger cans. Most of the operations are carried on by machine The sterilization is sometimes done in the ordinary canners' retort, or the cans n be placed on an endless conveyor, dipping into water or brine of a proper t perature, the speed of the conveyor being so regulated that the cans are sufficien heated to sterilize them during the passage.

Peas.—In the pea-canning industry the vines are cut with a mower, loa onto racks like hay, and hauled to the vining machines. The viner is a mach consisting of an outer and an inner cylinder revolving in opposite directions, inner one bearing paddles or beaters so arranged that as the vines pass through machine the paddles break open the pods. As the peas are thrown out, t pass through perforations in the outer cylinder, while the vines are discharged the opposite end. The shelled peas are next washed to remove all dirt and the mucous substance from the surface, thus insuring a clearer liquor in the c Grading for size is done by passing the peas over sieves, or into a revolving cylin having four sections with perforations of different sizes. The peas are next blanc in hot water to remove the mucous covering and to drive water into the peas that all will be tender. The time of blanching varies from one-half to five or m minutes, large mature peas requiring more time for the blanching than smaller o After blanching, the peas are filled into the cans by special machines, the cans filled with "liquor" consisting of water, salt, and sugar, sealed, and steriliz The time of processing varies, the average being 115° for thirty to thirty-five minu for the ordinary sized can.

Fruits.—The essentials in the canning of fruits do not differ from those vegetables. Stone fruits may be canned either with or without the pits. In case of such fruits as cherries, or other acid fruits, the tin can is coated on the ins with a laquer or enamel which protects the tin from erosion by the action of acid juices. The time and temperature of processing of fruits is usually less t that required for vegetables, for the reason that in the presence of the fruit aci the organisms are more easily destroyed than in foods in which acids are not prese

Controlling Factors in Successful Canning

Cleanliness.—Too much emphasis could hardly be placed upon he importance of cleanliness throughout the whole preserving process, nd especially in the preparation of the product for preserving. Vege- ıbles that have come into contact with the soil are pretty certain to arbor many spores of bacteria, and if as many of these are removed s possible by a thorough preliminary cleansing, sterilization may be ffected with greater ease and certainty. The necessity of cleanliness n the part of factory employees is needful only of mention, not only om the esthetic standpoint, but also from that of good health.

The Soundness of Raw Material.—The necessity of sound and holesome raw material is fully as great as that of cleanliness in andling. Foods are never better than when they are fresh. It makes o difference how long nor by what method they may be cooked, the uality cannot be bettered, and if food is unsound when put into the ontainers for canning, it will never be wholesome for food; and this ct is equally true whether the unsoundness is the result of diseased onditions of meats, fruits, or other products, or whether it is due to rdinary decay.

Water Supply.—Another essential for the success of the canner is n ample supply of pure water. It is a well-known bacteriological ct that outbreaks of spoilage have occurred in canneries, which could e traced to organisms getting into the goods from the water supply.

Receptacles.—The commercial canner recognizes two essentials for itable containers for his goods. First, they must be tight, both to revent the escape of the contained material and the entrance of con- ıminating organisms. Second, they must be of a material which will ithstand erosion or corrosion for a reasonable length of time, without iving up any notable quantity of foreign material to the food with hich they may be in contact. Glass is most satisfactory from this onsideration, but for reasons previously stated it is impracticable for se on a commercial scale. The difficulty from erosion in tin cans has een largely overcome by the use of enamelled cans as mentioned above.

Degree of Heat Required.—The actual sterilization of food roducts after placing in the containers is termed processing by the ommercial canner, and he appreciates fully that upon the care with hich the processing is done depends the success of the entire pack.

The degree of heat necessary to accomplish sterilization varies co siderably with different products.

One factor lies in the chemical composition of the fruits or vegetabl to be sterilized. It is, for example, well known that peas and asparag are rendered germ-free with much greater difficulty than beans, no withstanding the fact that the heating of the can contents of the form is accomplished much more easily than that of the latter. The high acid content of the beans facilitates the sterilization, and this same pri ciple holds true in a broad way for all products of an acid characte The canner and the housewife have long known that tomatoes we easy to preserve as compared with other vegetables. The canner al finds a variation from season to season. In some seasons the ac content of fruit and vegetable products will be higher than in other and consequently a lower processing temperature will suffice f sterilization.

In sterilization under pressure, as in the canners' retort, it is impo tant that the steam forced into the autoclaves should completely di place all the air, for otherwise at a certain pressure the correspondi temperature will not be obtained. Large cans require a longer time f thorough heating than small cans; closely packed cans are heated wi greater difficulty than loosely packed ones; the inner temperature frequently lower than that of the outer parts of the can.

In addition to these factors, the canner must consider the possib presence or absence of bacterial spores, which may gain entrance to h factory, and necessitate a higher temperature than that usually en ployed to accomplish the desired result.

Home Canning of Foods

The successful canning of foods in the home depends upon t same principles as those employed in commercial canning, namel cleanliness, soundness of raw material, and complete sterilizatio Aside from the universally used open kettle method of handling t foods, two other methods are now widely used: the cold-pack metho and the vacuum-seal method. In the cold-pack method, the produc are packed cold in their fresh natural state into the glass jars or oth containers. To the fruits, hot syrup is applied; to the vegetables an greens hot water and a little salt is added. Then the sterilizatio is done in the jars after they are partially or entirely sealed. Steriliz

on may be effected by the intermittent or fractional method of heating
n each of three successive days. The time of heating would depend
n the nature of the product, and the size of the container. The extra
me, labor, and fuel required for this method makes it impractical
here a large amount of canning is to be done. The more common
ethod is to sterilize in one period of heating by immersion of the con-
iners in water which is then brought up to the boiling point; or by the
e of steam pressure outfits of which the market affords several
pes. These operate on the same principle as the laboratory autoclav.

Spoilation of Canned Foods

Microbial changes occur when the goods have not been proc-
sed at a temperature sufficiently high to destroy all the organisms
hich may have been present in the uncooked food. In some instances,
ie organisms decompose the contents of the can with formation of gas,
using bulging of the ends of the cans sometimes to the point of burst-
g at the seams. Such cans are designated at the factory as "swells."
n other instances, the bacteria in the imperfectly sterilized cans cause
n acid fermentation with consequent souring of the contents. The
nner terms such cans "flat sours."

Detection of Spoiled Goods.—In cases of spoilage accompanied
y gas production, detection of the spoiled cans is easy from the
ulged appearance of the ends of the cans. On account of the exhaus-
on of air from the cans during processing, the ends of sound cans should
e slightly concave. If the ends of the cans are convex, it indicates
me abnormal condition of the contents, and such cans should be
jected. In the case of sours, detection is not so easy. The can may
ppear normal, and there may be no change in the contents apparent
) the eye on opening the can. Taste, however, reveals a more or less
ronounced disagreeable acid flavor. Canned meats, fish, or crus-
aceans are likewise liable to spoilage if sterilization has been im-
erfectly carried out. In these goods the change is generally but not
ways accompanied by gas production, hence detection is easy
ecause of the swelled appearance of the cans.

Disposal of Factory Refuse

The disposal of factory refuse has at times become a serious problem for the
mmercial canner. Of late years, however, methods have been devised for utiliz-

ing much of the material that formerly was allowed to accumulate about the factory in fermenting heaps to the extent of sometimes becoming a nuisance to the neighborhood.

At pea canneries several methods of utilizing the vines are in use. They may be converted into silage, either by putting into silos or stacking in large stacks. In some sections the vines are cured for hay. They are also valuable as a fertilizer.

Corn husks and cobs are also used for silage. Experiments were made by the United States Department of Agriculture in regard to the feasibility of using the refuse from the canning of corn for the production of alcohol. It was found, that on account of the expensive machinery and apparatus required in the manufacture a small factory could not profitably utilize the corn waste for alcohol. It was shown that where several factories were located within a short radius of each other by shipping their waste to a central plant, it might be used up to advantage.

Apple cores, "chops" and peelings are usually either used for vinegar making, or are made up into apple jelly. From one factory visited by the writer, the apple cores and peelings were dried, baled, and shipped to Europe, "to be made up into champagne."

Peach pits are sometimes sold to nurserymen for seed. Sometimes the pits are cracked and the meats used for almond meats. In many factories, no use is made of the peach stones.

In the classes of foods in which the waste is not large, the refuse is either hauled away to a dumping ground near the factory, or is taken away by farmers for its manurial value.

CHAPTER III*

THE PRESERVATION OF FOOD BY COLD

Introduction

In recent times cold storage has become of very great importance i the preservation of perishable food stuffs, and foods preserved by d usually command a higher market price than those preserved by er methods. This is probably due primarily to the fact that the neral appearance of refrigerated food resembles that of the perfectly sh article, in many instances very closely. Moreover, in many i tances cold storage, for a reasonable length of time, preserves not dy the appearance and the nutritive value, but also the chemical mposition, and even the delicate flavors of the original articles, so portant in determining market value. The great economic impor- nce of this industry is at once apparent, for it aims to preserve un- anged the over-abundance of one locality for transportation to other, and the over-production of one season of the year for subse- ent use.

The Effects of Refrigeration upon Foods in General

The decomposition of foods depends upon the activity of their own trinsic enzymes to some extent, but more especially upon the activity foreign microörganisms—bacteria, yeasts and molds. Cold acts as preservative, not by destroying these microbes, but by retarding or hibiting their activity. In general, cold not only retards the growth the microörganisms but delays their death also, tending to preserve em as well as the food unchanged.

In discussing the refrigeration of foods we may consider three periods treatment, (1) the removal of the heat or chilling of the food, (2) the olonged storage at low temperature, (3) the subsequent warming of e food before sale or consumption.

* Prepared by W. J. MacNeal.

Changes during Chilling.—The period of cooling is a relative short one, varying from a few hours to a few days in length. The chi physical change is the intentional removal of heat by conduction a convection, but there is usually also some loss of water by evaporatio If cooled to a sufficient degree the water content of the food m crystallize, altering to a considerable extent the physical structure the food substance (frozen food). Most foods are either actual living when chilling begins, or they are only recently dead and vario chemical changes due to intrinsic enzymes continue at a diminishi rate as the heat is removed. Decomposition changes, due to microb may also be in progress and continue during the process of chillin At this time the microbes living in the cold-storage chamber gain acce to the newly arrived food and others are added in the process of handlir The extent to which these will grow and multiply depends upon th ability to flourish under the storage conditions. In general the bacte which flourish at ordinary temperatures, producing the familiar deco position of the particular food, are greatly retarded in their activiti and other kinds outstrip them under the new conditions. The chang taking place during chilling are very important in some special i stances, and often a very definite procedure must be followed to obta a satisfactory result.

Changes during Storage.—This is often a relatively long peri and causes acting very slowly may ultimately produce marked alter tion. There is ordinarily some loss of water by evaporation, as w as the evaporation or diffusion of other volatile constituents, some them at times important factors in the flavor of the food. Other vol tile substances may be absorbed from the air of the storage roo The chemical changes of the chilling period continue at a greatly dimi ished rate, or may be entirely inhibited if the food is frozen. T behavior of the microbic content of the food is the most importa factor to be considered during this period. Besides those alrea present, various other microörganisms, bacteria, yeasts or molds, m gain access to the food from time to time, either from the circulati air or by contact with other things. The fate of the implanted microb will depend upon their nature and adaptation to the conditions exi ing in the stored food. Many of them perish, but many also survi the entire period of storage, and some may actively multiply. The can no longer be any doubt that some bacteria can grow at the te

rature of zero, and many kinds multiply at a fraction of a degree ove that point. In order definitely to inhibit microbic activity, the od must be frozen. When it is not frozen, bacteria continue to multi- y slowly at the lowest temperature of storage, and small variations i the temperature and in the humidity of the atmosphere serve to celerate their activity. Such variations also accelerate diffusion rrents in the food substance and so tend to distribute the microörgan- ns and their products. The extent of the resulting chemical changes i the food will depend upon these factors and upon the nature of the od, the temperature and the length of the period of storage.

Changes after Storage.—This is a relatively short period, but in ny instances a very important one as regards change in the food. warmed too rapidly, vigorous currents may be set up in the food ss by the great difference in temperature between the outer portion d the interior, serving to distribute microörganisms and their oducts. In the case of frozen foods rapid warming fails to restore e original physical structure. Dry cold foods are likely to condense isture from the warmer atmosphere unless it is particularly dry, d this condensed water becomes another cause of diffusion currents. frozen foods the water, in melting, may fail to reënter the food struc- re, and exude and drip away, carrying a portion of the soluble con- tuents with it. At this time still more microbes are likely to be added the food, and, together with those already present, they multiply th increasing rapidity as the temperature rises. As they may be eady pretty well distributed throughout the mass of the food, the ulting chemical decomposition is the more rapid. It is well recog- ed that, in keeping qualities, foods removed from cold storage are ch inferior to the corresponding fresh foods.

Refrigeration of Certain Foods

Meat, Fish and Poultry.—Meat, in this sense the flesh of mmals, is preserved by cold in two ways, by storage above the ezing-point (chilled meat) and by storage at $-10°$ to $-4°$ (frozen at). Fish and poultry are usually frozen for storage, often in the drawn condition.

Mammals killed for chilled or for frozen meat are slaughtered and efully dressed. For chilled meat the temperature is reduced by

storage in a cold air chamber to about +2° in forty-eight hours, an the meat is stored at a temperature between +1° and +2°. Unde these conditions the enzymes of the dead flesh continue to act an bacterial decomposition proceeds slowly, bringing about a process (ripening which, up to a certain point, improves the market value of th flesh by making it more tender and giving to it a more desirable flavo The extent to which the slow bacterial decomposition may proce before the flavor becomes disagreeable varies with different tastes, bu in general the beginning of proteolytic change which follows after th almost complete fermentation of the muscle sugar may be said to mar the desirable limit. This point is reached in from a week to thre months, depending upon the condition of the animal, skill and ca in slaughter and dressing, especially the extent of bacterial contamin tion at this time, and the accurate control of the storage condition In the production of frozen meat the carcasses are rapidly chilled i an air chamber at −20°, where the meat remains until frozen soli It is then kept at a temperature below −4°. Freezing produces marked change in the finer physical structure of the meat, as the wat crystallizes, leaving the protein material, with which it was former intimately mixed, in a shrunken and shriveled state between th crystals. Enzymic and bacterial activities are practically if not al solutely suspended under these conditions, and, save for slight surfa evaporation, such meat remains unchanged for long periods. Th subsequent thawing presents certain difficulties and requires particul care. If warmed very slowly the melting water crystals are imbibed b the protein material and the original structure of the flesh almost con pletely restored. The warmer air must be dry and must be kept i motion to avoid condensation of moisture on the exterior of the thaw ing meat. Bacterial activity is likely to gain considerable headwa during this process and the penetration of the microbes into the fle is favored by the diffusion currents. The more prolonged the warm ing process the greater the opportunity for bacterial decompositio Ordinarily, in order to avoid this, the thawing is carried out rapid and the finer structure of the meat is not restored. It is softer, darke and more moist than fresh or chilled meat, and usually sells at a low market price.

Fish and poultry are usually frozen for storage. As these foods a especially subject to rapid objectionable decomposition changes they a

rapidly chilled by immersion in ice water or packing in ice immediately after death, and are frozen as quickly as possible. During storage in the frozen condition microbic activity is suspended, but in the subsequent thawing the same physical and biological changes occur as in frozen meat. When fish and poultry are stored in the undrawn condition there is an abundant supply of bacteria at hand in the intestinal contents ready to multiply energetically during the chilling and thawing stages. It would appear desirable that the poultry should be killed and dressed with great care previous to freezing and that the period of chilling should be shortened as much as possible. Practically, however, it has been found that the dressing of poultry, as ordinarily done, previous to storage leads to such an extensive soiling of the edible flesh of the birds that their condition at the end of the storage period is often less satisfactory than that of undrawn frozen poultry, not only in gross appearance but also in respect to microbic content and chemical composition. Most frozen poultry is, therefore, stored in the undrawn condition.

The tendency of such food to undergo decomposition after thawing should be clearly recognized. Its sale as fresh or as chilled food is a fraud upon the purchaser. In fact many individuals seem to be peculiarly liable to suffer digestive disturbances after eating frozen poultry and such persons should avoid its use.

The nature and source of the bacteria which produce poisonous changes in poultry are not definitely known, but there is some evidence indicating that they belong to the para-colon group and that they are derived from the intestinal contents of the fowls.

Eggs.—The cold storage of eggs is an industry which has attained large proportions in recent years. A very constant storage temperature between $+0.5°$ and $+1°$ is essential for the best results. The humidity of the atmosphere is also of very great importance, as a dry air causes extensive evaporation from the egg and a too moist air favors the development of microörganisms on the exterior of the shell and the absorption of their products and even their penetration into the egg. A constant humidity of 70 per cent saturation has been found to be the best. Storage at this temperature and humidity greatly retards the growth of microörganisms and definitely inhibits the ordinary putrefaction of eggs. The activity of the intrinsic enzymes of the egg are not necessarily inhibited by this temperature, nor is the growth of all

microörganisms prevented. Unquestionably there is a marked dif
ence between the ordinary cold-storage egg and the strictly fresh
but to what extent this deterioration may be due to errors in stor
such as inaccurate control of temperature and humidity, use of od
erous crates for packing, decomposition changes previous to stor
too rapid chilling of the eggs, or too rapid warming of them a
removal from storage, and to what extent it is inherent in the n
perfect cold-storage procedure, is still somewhat uncertain. Do
less a certain amount of deterioration, especially the loss of the pecu
flavor of the fresh egg, is unavoidable in any method of prolon
storage. The discrimination in price in favor of new-laid eggs in
market is an indication of difference in actual value, and the sal
cold-storage eggs for new-laid or strictly fresh eggs is generally re
nized as a fraud by the purchaser and doubtless will in time b
recognized by law. The cold-storage egg is nevertheless a very valu
food and the economic importance of saving the over-abundant su
produced during the spring for use during the colder season of the
makes this industry a great benefaction to the public. Suitable reg
tion may be expected to remove its objectionable features.

Milk and Butter.—Milk as ordinarily sold at retail is not sub
to sufficient seasonal change in market price to make its prolo
storage advisable. But milk is so rapidly changed by bacterial acti
at ordinary temperatures that efficient dairy methods necessarily
clude prompt cooling of the milk after it is drawn from the ani
and the maintenance of a low temperature until it is delivered to
consumer. At the low temperature bacteria slowly multiply, u
the milk is actually frozen, but at a temperature slightly above
freezing-point very clean milk may be kept in perfect condition f
week, and it may be kept sweet for several weeks. Refrigeratio
milk cannot compensate for unhealthy animals producing it, nor
careless and uncleanly methods of handling. The cold does
destroy the microbes in the milk but only retards their multiplica
and chemical activity. In practice, especially in the transportatio
milk into large cities, it is frequently most economical to freeze the
and trust to insulation and the latent cold in the ice to maintain a
temperature during transportation. Such milk should arrive a
destination in a partly frozen condition.

The cold storage of butter is essential even when it is kept for

short periods, and the seasonal variation in price is sufficient to warrant its storage from summer to winter. The keeping qualities of butter depend upon many factors,* and the most efficient cold storage cannot compensate for previous deficiencies. In refrigerated butter there is a gradual diminution in the total number of living bacteria, with possibly a multiplication of a few particular kinds. There is a slow increase in acidity. In frozen butter the bacterial content and the chemical composition remain practically unchanged.

Fruits and Vegetables.—These foods are for the most part adapted to preservation for short periods at ordinary temperatures, and cold storage at a temperature slightly above zero is very effective in diminishing the rate of change in them. The humidity of the storage chamber should be kept constant at about 60 per cent saturation in order to diminish evaporation as far as possible without favoring the development of molds. These foods generally remain alive during storage and the changes due to intrinsic enzymes are often important. Some fruits need to undergo further ripening in storage before they are ready for consumption and this change may be accelerated or delayed by changing the temperature of the storage chamber. The development of bacteria and molds with consequent rotting is best delayed by maintaining dry clean fruits and vegetables in an atmosphere of very constant humidity and very constant temperature slightly above the freezing-point.

Legal Control of the Cold-storage Industry

At present there is a rather widespread prejudice against cold-storage food products, and in some respects this is not without justification. Cold storage preserves so well the external appearance of fresh foods that deception in the sale of them to the consumer is too frequently practised. This is extremely unfortunate for all parties concerned in such transactions. The proper branding of all cold-storage foods, clearly indicating their character and the length of time held in storage would ultimately benefit the producer, the consumer and also the cold-storage industry. Where cold storage is efficient such a practice would proclaim its efficiency. Where it is inefficient the cold-storage industry can ill afford to allow the consumer to be deceived concerning

* See chapter on the microbiology of butter.

the food he is purchasing. The strict enforcement of laws compelling the proper labeling of such foods and prohibiting their sale except when branded as such would quickly remove unjust prejudice against cold storage, and would place this industry upon a secure foundation, greatly increasing the possibilities of its service to the food producers and consumers, and at the same time promoting the legitimate interests of the cold-storage industry.

CHAPTER IV*

THE PRESERVATION OF FOOD BY CHEMICALS

The addition of preservative substances to foods is a very ancient practice, and as no extensive equipment is required it is one of the cheapest ways of preserving food, especially on a small scale. The resulting alteration of the food in appearance and composition is greater than when it is preserved by cold storage, for the preservative substance added becomes a more or less permanent constituent of the food, but the changes are not necessarily undesirable. The addition of chemical preservatives is often practised in conjunction with desiccation or cold, or sometimes even in canned or bottled foods sterilized by heat. All the substances employed as preservatives owe whatever efficiency they may possess to their ability to restrict the activity of microörganisms, that is, their antiseptic properties.

The Effects of Preservatives upon Foods in General

In only a few instances are chemical preservatives added to foods to be sold as fresh foods, and these practices are generally regarded with disfavor. Their most important use is in the prepared foods, the preservative being incorporated with the food during the process of preparation for storage.

The Process of Curing.—The procedures employed necessarily vary with different foods. Physical alterations in the food, such as changes in form, texture and water content are usually involved, as well as the solution of the preservative in the juices of the food. Chemical changes due to the intrinsic enzymes of the food, to the various accessory procedures such as drying, cooking or soaking in pickling solution may produce marked alteration. In some cases the preservative reacts chemically with some constituent of the food. During the curing process microbic activity may be more or less

* Prepared by W. J. MacNeal.

prominent at various times, playing its part in the chemical change. Bacteria, yeasts and molds are likely to be introduced into the food b the various manipulations and some of these may find conditions favo able for their proliferation. In some instances the activity of certa kinds of microbes appears to be essential to the proper curing a subsequent adequate preservation of the food; the preservative, t constituents of the food and the microörganisms mutually reacting bring about the desired result. It is worth noting that the add chemical preservative is never sufficiently potent to destroy wi certainty pathogenic microbes which may be present in the food.

The Period of Storage.—Unless the food has been sterilized a stored in sealed containers, slow changes in water content, in physic appearance and in chemical composition usually take place duri storage. The added preservative may continue to react with the fo substance and its decomposition products. During this period the is relatively little intimate manipulation of the food and therefore lit opportunity for the penetration of new microbes. Some of the already present may continue their activities at a diminished ra producing slow chemical changes often of a desirable nature rather th otherwise. Accessory conditions, such as desiccation, cold stora or sterilization and sealing, may greatly retard or check altogeth microbic activity.

The After-storage Changes.—The immediate preparation preserved food for consumption is frequently important. The p servative may be largely removed mechanically, or extracted wi water. During cooking peculiar chemical reactions may occur, a cooking is also important in the destruction of microörganisms rema ing alive in the food up to that time.

The Chemical Preservation of Certain Foods

Meats and Fish.—The preservation of meat and of fish by salti depends upon the increase of osmotic tension in the food, a physi change sufficient to prevent or greatly delay the growth of mic örganisms. Sodium chloride (NaCl) probably owes its preservati value solely to this physical effect. In practice its action is often su plemented by the addition of a small amount of saltpeter (KNO and sometimes also cane sugar ($C_{12}H_{22}O_{11}$). The fluids of the fl

a in part removed by this treatment, carrying away a part of the sluble constituents. The fluids which remain contain the added pservative substance in solution, and the whole mass of food subsnce is permeated by them. Potassium nitrate (saltpeter) reacts wh the flesh, being reduced in part to nitrite. This enters into a cbination with the coloring matter of meat, which upon cooking pduces the characteristic red color of meat cured with saltpeter.

The various manipulations during the process of pickling or dry cing serve to introduce numerous microörganisms. Many of these my flourish in the pickling fluids, but in a sufficient concentration of sa and at a sufficiently low temperature, decomposition ordinarily does n progress so as to become objectionable, and proteolytic decompsition (putrefaction) is effectually prevented. This protection of th protein depends to some extent upon the acidity of the medium, wch in turn is due largely to the bacterial decomposition of the mscle sugar. The powerful putrefactive bacteria (*B. œdematis* gup) flourish only in an alkaline medium. On the other hand too hn a degree of acidity becomes in itself objectionable on account of th sour or rancid taste, and it is, therefore, important that the acidpducing bacteria should be held in check somewhat. In practice, sapeter has proved of value for this particular purpose, and its acon apparently depends upon the antiseptic effect of minute quanties of nitric acid (HNO_3) and nitrous acid (HNO_2) set free from the sa by the excess of organic acids produced by the bacteria. The cng of meats by pickling solutions is often supplemented by desiccati and impregnation with the antiseptic substances of wood smoke.

The dry-salting of codfish is an example of preservation by increasing the osmotic tion. The fish is cleaned and beheaded, split longitudinally, and the vertebral comn removed. It is then carefully washed, and all visible blood is removed. The pies are next covered with dry salt and packed in open casks. The salt rapidly exacts water from the flesh and a strong brine results. After a few days the casks aremptied out, and the pieces of fish, now smaller because of the loss of water, are agn thoroughly washed and again packed in dry salt so that the adjacent pieces of fis are completely separated by an intervening layer of solid salt. The contents of e cask are subjected to high pressure to remove air, and the cask is finally closed.

The curing of hams is an example of preservation by increased osmotic tension cobined with the addition of chemical preservatives. After slaughter and chilling thams are injected with a solution containing 25 per cent common salt, 15 per cent grulated sugar, and 12 per cent saltpeter, and are then stored at a low tempeture, preferably between 0° and +4°, in a brine containing about 20 per cent

common salt, 5 per cent sugar, and 1 per cent saltpeter. The brine is renewed or or twice at intervals of a week or ten days. After about a month the hams a washed in warm water, dried and hung in wood smoke for several days. They a then stored in a cool place. The proportions of the various constituents of t pickling solutions are subject to rather wide variation, and in general it may said that the higher the temperature of the storage room, the more concentra must be the pickling solutions to insure satisfactory preservation.

Dairy Products.—Butter is usually salted with sodium chlori to impart the desired taste, and this salt also acts to some extent a preservative by increasing the osmotic tension of the moisture rema ing in the butter. Antiseptics such as boric acid, saltpeter, salicy acid and formaldehyde have been employed in the preservation butter, the first-mentioned appearing to be the most satisfacto One half of 1 per cent of boric acid incorporated with high-grade but previous to storage greatly delays rancid change.

Fresh milk and cream are also sometimes treated with antisept such as formaldehyde, but the use of any chemical preservative wh ever in these dairy products is unnecessary and generally disapprov.

Prepared Vegetable and Fruit Foods.—These foods are sor times preserved by vinegar, sugar or alcohol, the presence of which is f course very evident to the consumer. Other substances less rea y detected, such as sulphurous acid and sulphites, boric acid, salicylic a , benzoic acid and sodium benzoate, and formaldehyde, are also e ployed in foods which must be kept some time after exposure to the . These substances are incorporated with the food before it is pack , and serve to prevent the activity of microörganisms which gain ac s to it.

The Nutritive Value of Preserved Foods

The nutritive value of a food depends upon the amount of utiliz e food principles it contains. The food-principle content can be rea y measured by chemical analysis, and in general there is no import t difference between a preserved food and the corresponding fresh f d in this respect. The utilization of the food principles, howe r, depends upon a number of factors and may be greatly influenced y individual peculiarities of the consumer. One important factor in e utilization of a food, and probably the most important factor in de r- mining its market value, is palatability. In general, preserved fo ls

e pleasant to the taste when eaten at intervals, but upon prolonged
ily ingestion, the appetite for them fails and they may even become
tasteful. It would, therefore, appear to be erroneous to regard pre-
ved foods as in every respect as valuable from the standpoint of
trition as the corresponding fresh foods. The difference is not de-
ndent upon a change in the food-principle content, but must be
ght rather in slightly altered composition of the food and the
cific effects of newly formed substances, and especially in the
ssible effects of the continued ingestion of the contained chemical
eservatives upon the consumer.

The Effects of Food Preservatives

The essential characters of a food preservative include antiseptic
ion to prevent decomposition of the food, and absence of evident
sonous or deleterious influence upon the consumer. It follows there-
e that the effects of food preservatives upon the consumer, if they
st at all, are at any rate not easily recognized, and on account of the
nomic importance of the questions here involved this field of
ntific research has been energetically cultivated by investigators
h different viewpoints, and the results of investigation have been
cussed with some heat. The passage of the U. S. Food and Drugs
t was followed by considerable discussion of these questions. Gradu-
y the practical administration of the law has become more settled
d the use of many food preservatives is still permitted.

Substances Which Preserve by Their Physical Action.—The
eservative effects of sodium chloride seem to depend entirely upon the
h osmotic tension of strong salt solutions, and the same may be said
cane sugar. When diluted so as to be eaten with relish, these
bstances are themselves properly classed as foods, without deleterious
ects upon ordinary individuals.

Substances Which Preserve by Their Chemical Action.—
ese preservatives inhibit the activity of microörganisms in a dif-
ent way, not by withdrawing water from the microbic cell, but by
ering into chemical combination with the living substance in such a
y as to hinder its activity, or by entering into chemical reactions
h the food to produce new substances capable of attacking the
crobic protoplasm in this way. The ideal chemical food preservative

would be one which, without altering the food substance, would ex
hibit this poisonous property toward living protoplasm until the foo
was ready for consumption, and then would suddenly and permanentl
lose this property. None of the ordinary food preservatives approach
this ideal very closely.

Inorganic Food Preservatives.—Boric acid and borax are wea
antiseptics, practically a saturated solution of boric acid being nece
sary to inhibit ordinary bacterial growth. When employed as a dr
powder on the surface of meats, boric acid prevents the growth of mol
and most of it is removed from the food before consumption. Whe
incorporated with butter it is eaten, and 0.5 to 1.0 g. may be take
daily in this way alone. The effect of such amounts of boric acid upo
the consumer is still a disputed question. Wiley,* after an extensiv
investigation, concluded that small doses of either boric acid or bora
continuously administered for a long period create disturbances
health.

Nitric acid and nitrous acid and their salts are food preservativ
of some theoretical interest because it is well known that some bacter
readily decompose fairly strong solutions of nitrates, and also oxidi
or reduce nitrites. Apparently, however, this is true only in neutr
or alkaline solutions, and in the presence of free acid the activity
these microbes is quickly inhibited. The preservative effect of nitrat
and nitrites is best ascribed to the liberation of minute quantities
free nitric and nitrous acids from these salts, and these substances a
without value as preservatives in foods which are alkaline in reactio
The effects of the ingestion of nitrate or foods preserved with nitra
upon the consumer has been investigated by Wiley, who conclud
that the deleterious effects are slight and less clearly detected than
the case of the other preservatives. Minute but variable amounts
nitrites occur in foods preserved with nitrates, but whether the
amounts are sufficient to produce the specific nitrite effect upon t
blood circulation of the consumer has not yet been definitely ascertaine

Sulphurous acid and the sulphites are rather extensively used
chopped meat (Hamburg steak) and in cider and wines. The additi
of sulphite to chopped meat serves a three-fold purpose, retarding ba
terial decomposition, producing a red color on the exposed surface, a
removing odors of decomposition. It thus not only delays decompo

* U. S. Dept. Agr., Bureau of Chemistry, Bull. No. 84, Part I.

tn, but also to a certain extent conceals the decomposition which has eady occurred. The ingestion of moderate quantities of sulphites i food has at times been followed by acute gastric derangement in n, and prolonged feeding of meat containing sulphites has been llowed by inflammatory changes in the kidneys of experimental imals.

Fluorides have been used to a slight extent in beverages, but acute stric derangement and depression of the heart are caused by rather sall quantities, and probably on this account the salts of hydrofluoric d have not come into very general use as food preservatives.

Organic Food Preservatives.—Formic acid ($H \cdot COOH$) and etic acid ($CH_3 \cdot COOH$) are produced by microbic activity and their eservative action appears to depend more upon the degree of acidity tn upon the character of the acid radical. Both these acids appear t be utilized as food in the body of the consumer.

Benzoic acid and benzoates are rather extensively employed in pared vegetable food products, such as jams and catsups. The tiseptic effect seems to be due wholly to free benzoic acid, even where i s added in the form of the salt, but the action is not due merely to t acidity (*i.e.*, the hydrogen ion). Benzoic acid is not utilized as a f d in the body, but is excreted by the kidneys in the form of hippuric a d. It has been said to produce irritant effects upon the stomach a d the kidneys, and to arrest the action of digestive enzymes in dilute s utions, but the Referee Board* of the United States Department of Agriculture, after extensive investigations, concluded that small doses o sodium benzoate mixed with food are not injurious to health, and o not impair the quality or nutritive value of the food.

Salicylic acid and the salicylates have been used for much the same p rposes as benzoic acid, and there does not appear to be much dif- f ence between the two acids, either in their efficiency as preservatives o in their possible deleterious effects upon the consumer. Salicylic a d is more expensive. After extensive investigation Wiley† has o cluded that the addition of salicylic acid and salicylates to foods is r prehensible in every respect, this conclusion corresponding to the r ults of similar work by the same investigator‡ upon benzoic acid.

* U. S. Dept. Agr. Report No. 88, May, 1909.
† U. S. Dept. Agr., Bureau of Chemistry, Bull. No. 84, Part II, 1906.
‡ U. S. Dept. Agr., Bureau of Chemistry, Bull. No. 84, Part IV, 1908.

Formaldehyde is very efficient as an antiseptic, delaying microbia decomposition when added to foods in very small quantity. Its us for this purpose is generally condemned, partly because of its hardenin or "fixing" effect upon the protein constituents of the food, tending t make them more indigestible. Its use in milk and milk product though still practised to some extent, has been prohibited by law i some states.

Alcohol ($CH_3 \cdot CH_2OH$), in sufficient concentration, is an excelle preservative, but its presence in foods is readily detected, and it giv rise to characteristic effects upon the consumer. Furthermore, suc foods are subject to special taxation as alcoholic products. Its us as a food preservative is therefore limited.

Wood smoke has been employed for centuries in the curing of meat Its antiseptic properties probably depend for the most part upo creosote and pyroligneous acid, constituents of wood smoke which a antiseptic and also undoubtedly poisonous in sufficient doses. Smol ing is a time-honored custom, however, and the amount of the substances actually consumed with the smoked meat is doubtle exceedingly minute.

Substances Added to Foods to Improve the Apparent Qualit —Several chemical substances are employed in various foods to ir prove the appearance, or to simulate the taste of a higher-grade produc In some cases the presence of these agents is known to the consume and desired by him; in other instances they are employed to decei the purchaser. Butter coloring is quite generally used to produce t color of June butter the year around; nitrates bring about a pleasi red color in cooked pickled meats; copper sulphate is used to give more brilliant green color to prepared vegetable foods; sulphites resto the red color of freshly cut meat to meat far from fresh; sacchari devoid of food value gives a taste resembling sugar to a variety preparations at a great saving in cost to the manufacturer; carbonat of the alkalies or alkaline earths, added to milk, neutralize the aci resulting from bacterial decomposition and so keep the milk swe inorganic acids added to weak vinegar increase its acidity. Some these practices are so universal and so well known that they are longer criticized; others, such as the use of chalk in milk, are general disapproved.

Legal Control of the Preservation of Foods by Chemicals

The desirability of legal regulation of the use of chemical food preservatives is now generally recognized, but there is still considerable diversity of opinion concerning what this regulation should be. Few, indeed, maintain that a substance exerting antiseptic action upon microörganisms outside the body is wholly without influence, after ingestion, upon the enzymes and bacteria of the normal digestive tract, even if we disregard the possible effects of the substance after absorption. It seems necessary to grant the existence of some effect, even though it be so slight as to have escaped detection. Over against the possible injury to the consumer must be placed the economic saving through the use of the preservative, often involving a considerable amount of money. In the absence of accurate and trustworthy knowledge concerning the actual influence of preservatives in the human body it would seem wise to prohibit all deception in regard to their presence. The principle advocated by Pasteur (1891) would still seem to be best, that is, to allow the use of preservatives, which are not known to be dangerous, upon the condition that their presence and the exact amounts be definitely and clearly stated on an appropriate label for the benefit of the purchaser and the ultimate consumer. Such regulation would not only protect the consumer against deception and fraud but would go far toward removing unjust prejudice against preservatives, for even now there is little or no objection to those preservative substances of which the presence and the amount can be detected and roughly measured by the senses, such as salt, sugar, spices, vinegar and wood smoke.

CHAPTER V*

MICROBIAL FOOD POISONING

General Considerations

Illness following the ingestion of food, more or less definitel ascribable to the food, has been long recognized. The Mosaic regula tions in regard to foods forbidden to the Jews are evidently designed i part to avoid the occurrence of food poisoning. In recent times recog nized instances of food poisoning have been sufficiently frequent t make the subject one of considerable practical importance, but ther are undoubtedly many instances of actual food poisoning in which th causal relation of the food remains unrecognized or even unsuspected.

Food poisoning is usually suspected at once upon the occurrence o sudden acute illness in a number of people at the same time, after the have partaken in common of some particular food or foods. Th causal relation is especially evident when, as sometimes happens, large number of people are affected in the same way immediately afte eating together at a banquet, not having been associated with eac other either before or after the meal. When a smaller number of ind viduals is involved, the connection with food may be more obscure For this reason most of the well-authenticated instances of food poisor ing are instances in which many persons have been affected at the sam time. Acute food poisonings involving only a few persons probabl occur very frequently in the home, but they receive little public notic unless fatal, and are often dismissed as mere "errors in diet," or a "indigestion." A careful study of these cases is likely to be mad only where there is suspicion of criminal poisoning, or some othe practical end to be served by the investigation. Chronic forms o food poisoning are for obvious reasons very difficult to recognize wit certainty, and some of the forms of disease now regarded as due t chronic food poisoning may eventually prove to be due to other cause On the other hand chronic food poisoning may really be more impor

* Prepared by W. J. MacNeal.

nt than is recognized at present. The subject is still in a very ubtful state.

To establish by laboratory investigation the poisonous character of ods requires toxicological training and experienced judgment, a dis- ssion of which would lead beyond the scope of the present chapter. r a general review of this field of work and references to further formation the articles cited at the end of this chapter should be nsulted.

Several different classes of food poisonings may be recognized cording to the source of the poisonous substance.

The material of plants or animals may be naturally poisonous to an as a result of the physiological activity of their own living sub- ance. Poison of this kind may be constantly present throughout e tissues, or it may be confined to certain parts, or it may occur only particular times or seasons. Some instances of poisoning with fish nd with mushrooms belong to this class, and possibly also some of the stances of poisoning with potatoes of high solanin content.

Plants and animals may feed upon substances not poisonous to emselves, and these substances may remain a constituent part of eir bodies to poison man when consumed by him. Some poisonings ith freshly killed game are considered to be of this nature.

Any food may contain foreign poison added to it by design or by ccident, such for example as the salts of the various poisonous metals. he amount of tin or lead passing into solution in canned or tinned oods may conceivably be sufficient to cause poisoning, but there is no eliable evidence that it has ever occurred.

Animals may be infected with pathogenic bacteria or with other arasites capable of infecting man, and the use of food products from uch animals may cause disease. Tuberculosis, trichinosis, and apeworm may be acquired in this way.

Any food may serve as the passive carrier of infectious agents, such as *B. typhosus*, and some foods may even favor the multiplication of athogenic bacteria gaining access to them.

A food may undergo chemical changes due to microörganisms in- capable of infecting man, resulting in the production of poisonous sub- stances in the food. Undoubtedly the great majority of instances of food poisonings belong in this class. The bacteria causing these changes have been designated as pathogenic saprophytes.

The last three classes comprise the microbial food poisonings, ar these are the kinds of food poisoning with which we are at present mo particularly concerned.

Infections of Food-producing Animals Transmissible to Man

Animals dead of infectious diseases or slaughtered in the last stag of disease are not ordinarily used for food, nor is the milk of su animals ordinarily considered wholesome. This custom is certain an ancient one, and is doubtless founded upon observation of u favorable results following the consumption of such food. Exa knowledge of the nature of the diseases transmitted in this way is more modern development, and this more exact knowledge is nc being applied to some extent through food-inspection regulations prevent the transmission of such diseases.

Tuberculosis of cattle has been shown by Smith to be due to a ger somewhat different from that causing the ordinary human tuberculos and this discovery has called into question the necessity of avoidi the use of food products from tuberculous animals. After a co siderable amount of controversy it may now be regarded as definite established that the bovine type of tubercle bacillus is capable infecting man, and that a very considerable proportion of cases tuberculosis in children are due to this type of organism, the infecti probably arising through the use of milk from tuberculous anima Anthrax, glanders, actinomycosis, and acute enteritis of animals a also transmissible to man. Food products from animals afflicted wi these diseases should not be used until they have been passed upon competent authority. Further information concerning them will found in the sections dealing with these particular diseases.

The human disease known as septic sore throat may be due to i fection with streptococci present in cow's milk. Whether these virule streptococci are derived from an inflamed udder of the cow or from t throats of persons who handle the milk is not fully ascertained, b the inflamed udder is to be looked upon with suspicion.

In this connection it may be mentioned that some of the anim parasites, especially trichinæ and various sorts of tapeworms, ga access to the human body with the food. Thorough cooking usua serves to kill these parasites, as well as the pathogenic bacteria, b

dinary cooking should not be too implicitly relied upon to accomplish is result.

Human Infections Transmitted in Food

Food may serve as the passive carrier of the germs of any human infectious disease capable of indirect transmission upon dead material. n some foods, especially milk, these infectious agents may actually ultiply. Typhoid fever, diphtheria, and scarlet fever appear to be ither frequently disseminated through the agency of food, and paraphoid fever seems to be commonly transmitted in this way. Especial recautions are advisable to prevent persons afflicted with dangerously ommunicable diseases and those who are chronic germ-carriers from ngaging or continuing in occupations concerned with the immediate reparation of food for consumption, particularly such occupations as nilk producers, milk handlers, market-dairying, cooking, and serving ood. Numerous serious epidemics have been traced to such sources n recent years.

Food Poisoning Due to the Growth of Saprophytic Bacteria in the Food

Most food poisonings are due to food derived from perfectly healthy nd wholesome animals or plants, which has subsequently undergone ome bacterial decomposition giving rise to poisonous products. Our knowledge of the specific causes of the poisonous changes is, however, very incomplete, and on account of the difficult nature of investigation in this field, some of the conclusions reached by careful men are still open to question. The bacteria which have been most frequently identified with various epidemics of food poisoning are the following: *B. enteritidis* in meat poisoning; *B. botulinus* in meat and in sausage poisoning; *B. paratyphosus* in poisoning with meat, chicken, shellfish, and vegetables; *B. coli* in cheese poisoning and in milk poisoning; *B. vulgaris* in meat and in vegetable food poisonings. Doubtless other microörganisms, as yet unrecognized, play an important part in many food poisonings, and there is reason to believe that some of these important unknown forms are anaerobic bacteria.

Poisonous Meat and Sausage.—The flesh of a healthy animal is ordinarily free from bacteria at the time of slaughter, and bacterial

changes must begin at the surfaces of the pieces of meat and graduall extend inward. In diseased animals, bacteria more frequently circulat in the blood, and the flesh may be contaminated throughout when th animal dies of the disease or when it is slaughtered, not only with th specific germs of the disease but also with bacteria derived from th intestinal tract of the animal. It is a matter of observation tha the flesh of diseased animals is more liable to undergo early putrefac tive and poisonous changes than that derived from healthy animals Hashed meat is, of course, much more prone to bacterial decomposition because in it the bacteria have become well distributed throughou the mass, and ideal conditions are provided for the development c anaerobic as well as aerobic bacteria. Minced chicken and chicke pie appear to be very frequent sources of acute poisoning in the Unite States, and epidemics of sausage poisoning have repeatedly occurrec especially in Germany. The bacteria found to be concerned in thes instances have been *B. enteritidis*, *B. paratyphosus*, *B. coli*, and *E botulinus*. Some of these poisons, as for example the toxin of *E botulinus*, are rendered inert by boiling, but occasionally bacteria poisons which are not destroyed by such high temperatures may b present in food. Moreover, meat rendered poisonous by these bacteri may show no evidence of putrefaction. *B.* (*Proteus*) *vulgaris* ha also been found in some samples of poisonous meat, and this finding i usually associated with definite evidence of putrefaction.

The symptoms of meat poisoning are usually those of acute gastrc enteritis, vomiting, cramps, and diarrhœa. The patients often recove very quickly, but occasionally the illness is rapidly fatal, or it ma merge into a subacute form resembling or identical with paratyphoi fever. In those instances of poisoning due to the presence of *E botulinus* the symptoms are of a different kind, consisting almos solely of nervous disturbances, secretory and motor paralyses, withou fever, resembling in many respects poisoning with atropin. In thi form of meat poisoning the death rate is relatively high, about 40 pe cent of the cases ending fatally.

Fish poisoning is of two general kinds, that due to poisons natura to the fish, and that due to poisons formed by bacterial activity in th flesh of the fish. Blanchard has applied the Spanish name "Siguatera to the first kind and the term "Botulism" to the second. In th Japanese fish of the genus Tetrodon the roe is poisonous, giving ris

severe gastro-intestinal irritation and convulsions. The remainder the fish is not poisonous. In some other fishes the sexual glands re poisonous during the spawning season; others are provided with pecial poison glands connected with protective spines or barbs. These are examples of poisons natural to fish. Bacterial poisons are kely to be formed in any kind of fish, given the suitable conditions, nd thus give rise to the kind of fish poisoning designated as botulism. Cases of this kind have resulted from eating (spoiled) canned salmon nd sardines. Poisoning may also result from eating diseased fish, he effects being due to poisons elaborated by the infecting bacteria n the body of the fish before consumption. This appears to be a ather common form of fish poisoning in Russia. *B. paratyphosus* has een isolated from some poisonous fish, and certain toxicogenic an-erobes have been found in others.

POISONING WITH SHELL-FISH is so well recognized that this form of ood is not customarily used at all during the warmer part of the year, May to August inclusive, the months without an *r* in their names. *Shell-fish* may serve as carriers of human infectious diseases, such as typhoid fever; they may be poisonous on account of actual disease or through serious contamination due to living in dirty water; or they may be poisonous because of decomposition which has taken place after removal from the water. According to the symptoms produced, there appear to be at least three distinct varieties of shell-fish poisoning, one a purely gastro-intestinal disorder, the second an involvement of the nervous system with itching skin eruption and convulsions, and a third type resembling very closely alcoholic intoxication. The exact nature of the microbic agents concerned in these different types of poisoning is unknown. It is pretty well established, however, that the poisonous character of shell-fish is due either to their living for some time in dirty water, or to their too long preservation, especially at high temperature, after removal from the water.

MILK, ICE-CREAM AND CHEESE sometimes give rise to poisoning, and although these instances are small in number in comparison with the enormous amount of milk and milk products consumed, yet in the aggregate they are numerous. That many human infections may be transmitted by milk has already been pointed out. In the summer, milk is undoubtedly a great factor in the infant morbidity and mortality, and this poisonous action is largely due to bacterial changes in the milk.

Extraordinary precautions are therefore essential in the production an care of milk to be used as food for children, particularly during th warmer season of the year. Severe poisoning of adults with milk, ic cream, or cheese, is relatively less frequent. Cases which have bee studied have been traced to the development of *B. coli* or *B. paraty phosus* in these foods. There is some evidence that other bacteri probably strict anaerobes, are also sometimes concerned. Stric cleanliness, proper refrigeration, and pasteurization of milk of uncertai character, may usually be relied upon to prevent milk poisonin Ice-cream should be made only from wholesome materials and wit due regard to cleanliness in making it. The causes of serious chee poisoning are not definitely known, but such poisoning may be avoide to a large extent at least, by using only standard varieties of cheese the proper odor and flavor.

VEGETABLE FOOD POISONING, in an acute form, has followed the us of sprouting and partly decomposed potatoes, and also various canne vegetables, particularly those of high protein content, such as bean The large majority and possibly all of these cases are due to decompos tion changes in the foods, *B. botulinus* and *B. proteus* appearing to b the microbes most frequently concerned.

There are also certain definite, more or less chronic diseases whic have been attributed to the use of certain grains as foods. Ergotisn characterized by cachexia, gangrene, and convulsions, is caused b eating the fungus, *Claviceps purpurea*, which grows as a parasite upo rye. The grain of this parasite has a considerable commercial (medi inal) value sufficient to pay for its separation from rye where it occur so there is little economic excuse for food poisoning from this cause.

Beriberi or kakke is an acute or chronic nervous disorder whic has been observed especially in the Orient, Japan and the Philippin Islands, although it has also been found in Brazil, in Labrador an rather frequently among sailors after long sea voyages. At one tim the disease was ascribed to the use of fish as food, later to the use rice. Modern studies, especially those of Chamberlain, Vedder an their associates in the Philippine Islands, have shown that beribe may be prevented by including beans, unpolished rice or rice hulls i sufficient quantity in the diet and furthermore that those alread afflicted with the disease usually recover completely when given thes foods or when treated with an alcoholic extract of rice polishing

he curative principle of rice polishings has been studied by Funk who s named it vitamine. He ascribes the causation of beriberi to a lack this supposedly necessary vitamine in the food and this theory has en very favorably received. It must be acknowledged, however, at the etiology of beriberi is still not convincingly proven. The scovery of a remedy which eradicates a given disease is not sufficient prove that the lack of this particular therapeutic agent is the es-ntial cause of the disease.

Pellagra is a cachexia, characterized by a definite sort of skin erup-on, which has been ascribed to the use of maize (Indian corn) as od. This disease is discussed in a separate section (page 813).

The Chemical Nature of Food Poisons

The poisonous substances in foods are for the most part of the same ature as the poisons of the pathogenic bacteria. The simplest in ructure of these poisons belong to the alkaloidal substances, substi-uted ammonia and ammonium compounds, called *ptomains* (page 171). everal of these have been prepared in a pure state, for example, mytilo-oxin ($C_6H_{15}NO_2$) from poisonous shell-fish, and neurin ($C_2H_3 \cdot N(CH_3)_3OH$) from putrefied horse, beef, and human flesh. Although otomains undoubtedly occur at times in poisonous foods, they are not ow considered of so much importance in food poisoning as formerly, or in the majority of samples of poisonous food the search for ptomains as been in vain. The poisonous effects are believed rather to be due or the most part to much more complex bodies resulting from the arliest analytic changes in the food protein, or else to bodies built up by actual synthesis by the bacteria. Such substances are classed with the toxic proteins and the true toxins. Their chemical composition and structure are not definitely known.

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CHAPTER VI*

MICROÖRGANISMS OF THE DIGESTIVE TRACT

Introduction

The digestive tube of the vertebrate animal is in communicatio with the external world and is the passageway for a great variety materials constituting the food of the animal. This food brings wit it various sorts of microbes, at times in considerable numbers. With the digestive tube the food is more or less completely resolved by th processes of digestion into soluble nutritive split products, which furnis an excellent medium for microbic development. It is not surprisin therefore, that there is an enormous multiplication of microörganisn within the intestine, both in health and disease, and that this mult plication is most active during the digestion of the food.

Microörganisms of Certain Portions of the Alimentary Can

The entire digestive tract is free from microbes during norm intrauterine life. After birth the canal is quickly invaded by bacteri chiefly through the mouth and nose, but to a lesser extent also throug the anal orifice. In the mouth, pharynx and intestine, some of the invaders establish themselves to remain throughout the life of th individual host. The species of microbes present and the numeric proportions of the different species of normal buccal and intestin microörganisms vary somewhat with the age of the host and the chara ter of his food. They are also considerably disturbed sometimes b the entrance and multiplication of pathogenic germs, giving rise t disease in their host, such as *Oidium albicans* in the mouth or the cho era vibrio in the intestine.

Microörganisms of the Mouth.—The buccal cavity presen conditions of temperature, moisture, chemical reaction and a variet of food substances in its various parts, which are very favorable to th growth of many microbic species. Aerobic, facultative and anaerob forms are found and the species are very numerous. Miller, in a fe

*Prepared by W. J. MacNeal.

weeks, was able to isolate more than a hundred different kinds of bacteria from the mouth. Many of these are doubtless only transient residents, having gained entrance with food, water or air.

Among the almost constant inhabitants of the mouth may be mentioned the streptococci, both the variety which produces a green color on bloodagar, the *Strept. salivarius* or *Strept. viridans*, and the hemolytic variety, *Strept. hæmolyticus;* the *M. pyogenes var. aureus* and *albus;* the *Iodococcus magnus* and *Iodococcus parvus* of Miller, which may be cultivated upon a sugar-starch gelatin-agar medium and are stained blue by iodine; two or three species of spirilla, described by Miller, which may be cultivated with some difficulty upon ordinary nutrient agar; *B. fusiformis* of Vincent, which may be cultivated as a strict anaerobe in media containing blood serum or ascitic fluid; *B. maximus* (*buccalis*) of Miller, a bacillus forming threads 0.5 to 1.5μ wide and 20μ or more in length, cultivable upon maltose agar or potato gelatin; *Leptothrix buccalis*, a slender unbranched filament, which may be brought to development on ordinary media, with some difficulty.

Even more definitely characteristic mouth bacteria are those which are found in every human mouth (except in very young children) and which are not cultivable in artificial media at all or only under special artificial conditions never met with in nature. Among these forms may be mentioned the *Iodococcus vaginatus*, an encapsulated organism which may be stained blue by Lugol's solution acidified by addition of lactic acid; the *Sp. sputigenum*, which is found especially at the inflamed margin of the gums; the *Spirochæta buccalis*, *Spirochæta dentium*, *Spirochæta microdentium* and *macrodentium*, organisms which are found in the mucus about the teeth, but are especially numerous in denuded areas or in abscess cavities of the gums or in carious teeth. The spirochetes of the mouth have been successfully cultivated by anaerobic methods in serum and in ascitic fluid by several investigators, notably by Noguchi.*

The amœba of the mouth, *Entamœba* (*Endamœba*) *buccalis*, may be found in nearly every individual in the deposits between the teeth and especially in carious teeth. The cell is 6 to 32μ in diameter, actively motile, with few lobose pseudospodia. The nucleus of the living amœba is visible. Its food apparently consists of bacteria and the bodies of

* Noguchi, Journ. Exp. Med., 1912, XV, 81.

leukocytes. It does not appear to penetrate living tissue. Oth
mouth amœbæ have been described. Whether they really belong
species distinct from *Entamœba buccalis* is questionable. Recently
has been claimed that the amœbæ of the mouth bear a causal re-
tion to *pyorrhea alveolaris*, but the claim has not been convincing
proven.

The various characteristic buccal microörganisms are found
particular parts of the mouth and their numbers vary considerab
according to the cleanliness of the mouth and teeth, presence or absen
of denuded areas, ulcers, sinuses or carious teeth. The iodine-staini
varieties are especially abundant between the teeth and upon starc
food residues. The spirochetes, on the other hand, are more abunda
in the serum exuding from denuded areas and in pus cavities.
fusiformis (Vincent) is often found in normal buccal mucus but it
especially abundant in the necrotic ulcers of the tonsil in the disea
known as Vincent's angina, in which situation it is always associat
with numerous spirochetes.

Some of the members of the normal mouth flora occasionally pl
definite pathogenic rôles. There can be little doubt that the starc
fermenting forms produce acid, thus attacking the mineral matter
the teeth and favoring dental caries. The pathogenic rôle of *Strep*
viridans, when it penetrates into carious teeth, causing root absce
and, probably by metastasis from this focus, giving rise to arthritis a
endocarditis, is indicated by a mass of circumstantial and experiment
evidence which is well nigh convincing. The frequently serious natu
of infections with the hemolytic streptococcus are well known. Doub
less members of this variety of streptococcus in the mouth are read
to acquire virulence whenever lowered resistance of the host presen
a favorable opportunity for them to invade the tonsils, the pharynge
mucous membrane, the Eustachian tube and middle ear, not to mentio
more distant parts of the body.

Certain very specific pathogenic microörganisms are found in th
mouth and pharynx from time to time and they sometimes produc
lesions there. *Spirochæta pallida* is especially abundant in the bucc
and pharyngeal lesions of secondary syphilis. The tubercle bacill
is expectorated through the mouth in open pulmonary tuberculosi
The pneumococcus is often found in the mouth and pharynx, even i
health and is especially numerous and virulent in cases of lobar pneu

monia. The influenza bacillus and *Bact. diphtheriæ* are also occasionally found in the throats of healthy persons as well as of those suffering from the diseases to which they give rise.

MICROÖRGANISMS OF THE STOMACH.—The microbic flora of the healthy stomach consists almost exclusively of organisms swallowed. The gastric juice restrains bacterial multiplication and kills a large majority of the bacteria which enter the stomach. In diseased conditions the absence or reduced concentration of the hydrochloric acid may permit the multiplication of yeasts, of large lactic-acid bacilli (Boas-Oppler bacilli), of encapsulated cocci (*Sarcina ventriculi*), or even of flagellate protozoa, such as *Lamblia* and *Trichomonas*.

MICROÖRGANISMS OF INTESTINE.—The duodenum receives from the healthy stomach relatively few living bacteria. The secretions of the liver, pancreas and of the duodenal wall are very free from bacteria and they tend to flush out the duodenum. In health this portion of the intestine is quite free* from living bacteria in the intervals when food is absent and it contains relatively few bacteria during digestion. Among the living microörganisms most frequently found here are Gram-positive cocci which fail to liquefy gelatin. *B. coli* is uncommon. In spite of the negative results of culture work upon duodenal juice, it is always possible to see with the microscope abundant bacterial cells in it. These are probably dead.

From the upper end of the jejunum to the ileocecal valve, the number of bacteria in the small intestine progressively increases. In the intervals when food is absent, even these portions of the small intestine tend to free themselves from bacteria, in part, probably, because they are continually flushed out by the intestinal secretion, in part probably in part also, as has been maintained by Kohlbrugge,† because of a definite bactericidal property of the intestinal mucous membrane. However this may be, it is certain that living organisms of the *B. coli* group and various streptococci are commonly found in the intestinal contents taken from the jejunum or ileum at operation or at autopsy and that these organisms are quite numerous in the material discharged from the lower end of the small intestine in cases of ileocecal fistula.‡ The relative abundance of the different kinds of bacteria

* MacNeal and Chace, Arch. Int. Med., 1913, XII, 178.

† Kohlbrugge, Centrabl. f. Bakt. Abt. I, 1901, XXIX, 571; *ibid.*, 1901, XXX, 10; *ibid.*, 1901, XXX, 70.

‡ Macfayden, Nencki und Sieber, Arch. f. Exp. Path. und Pharm., 1891, XXXIII, 311.

may be altered by changing the character of the diet, a fact of imp- tance in the treatment of intestinal infections.

In the cæcum there is a sudden enlargement of the lumen of t intestinal canal and a consequent retardation of the movement f the intestinal contents. The blind pouch also favors stagnation. n this region the whole intestinal contents usually acquire a chemi l reaction neutral or alkaline to litmus. All these factors favor t enormous multiplication of bacteria. Indeed, the cæcum and t remaining large intestine constitute the great bacterial incubator f the healthy body. Here *B. coli* multiplies enormously; the str t anaerobes, *Bact. welchii* and *B. edematis* flourish under most favora e conditions. Various streptococci, staphylococci and spirochetes mu- ply either in the food residues or in the intestinal secretions. n easily digested mixed diet favors the facultative anaerobes, while exce- ive feeding of starchy foods and of meat leads to an overgrowth of e strict anaerobes, especially those of the *Bact. welchii* group. Many f these bacteria will then be found to stain blue with iodine, giving t so-called granulose reaction. A milk diet, especially if limited n amount and well digested by the individual, favors the micro-aeroph c *B. bifidus* of Tissier, the organism which is dominant in the fæces of t healthy breast-fed infant and occasionally very abundant even n adults.

In the lower portions of the large intestine, as a result of progress e absorption from the contents of the bowel, there is a concentrati n and overcrowding of the bacteria which have developed at higher leve . The vast majority of them die and these dead cells, together with t still abundant living microörganisms, make up about a third of t substance of the fæces. The fæces are composed of rejected fo l residues, residues of intestinal secretions, of bile and pancreatic ju e and abundant microörganisms, some of the latter still actively mul- plying, but the majority of them dead and in various stages f disintegration.

THE MICROÖRGANISMS OF THE FÆCES.—The microörganisms of t fæces represent the end result of the progressive multiplication or d- integration, or both, of the organisms originally present in the fo l together with all those added at various regions of the alimentary can . The microbic flora of the large intestine is, however, most promine t in the fæces. The total quantity and the proportions of the varic s

nds of microbes in the fæces varies considerably even in health, depend-
; upon various factors, among which age of the individual and
aracter of the food are very important.

The first meconium passed after birth may contain few or no micro-
ganisms. Within a few hours, however, they appear in the intestinal
charges. The earliest forms are usually large diplococci to which
soon added various bacilli, small diplococci and tetrads. Among
bacilli, a long slender form with a large oval terminal spore, the
adlet bacillus of Escherich, is particularly conspicuous. *B. coli* is
o present at this time and several gelatin-liquefying forms of bacilli
n be isolated in cultures, among them *B.* (*Proteus*) *vulgaris* and *B.*
btilis. Anaerobic cultures demonstrate the presence of *Bact. welchii*,
edematis and *B. bifidus.*

As the meconium is replaced by the residue of the ingested mother's
lk, the previously variegated bacterial flora suddenly becomes very
nple and during the whole period of exclusively breast feeding the stools
ntain enormous numbers the Gram-positive micro-aerophilic *B.*
fidus of Tissier, with only small numbers of *B. coli* and very few cocci.
ne dominance of *B. bifidus* may readily be demonstrated by making a
ries of dilution cultures in tall tubes of glucose agar, according to the
ethod of Veillon, and incubating them for five days or more. When
e child begins to take cow's milk there is a sudden increase in the
lative numbers of *B. coli* and streptococci and with the addition of
archy foods to the diet the fæcal flora gradually comes to resemble
at of the adult.

In the healthy adult taking a mixed diet, the fæcal flora consists for
e most part of Gram-negative bacilli of the type of *B. coli.* There are
so many diplococci, a few small Gram-positive bacilli (*B. bifidus?*) a
nall number of *Bact. welchii* and its free spores, a few representatives
the *B. edematis* group and a variable number of slender spirochetes.
erobic plate cultures on agar or gelatin often bring to development
ly *B. coli.* When a vegetarian diet rich in indigestible residue is
nsumed, the diplococci are much diminished in numbers; numerous
rge bacilli, *Bact. welchii* and *B. subtilis*, take their place. The con-
mption of excessive quantities of meat and starchy foods may lead
a considerable increase in the numbers of the *Bact. welchii* group and
me of the bacteria of this group may be stained brown or blue with
dine because of the granulose which they contain. The bacteria

normally present in the fæces are produced almost altogether by mul plication within the intestine. It is nevertheless possible for swallow organisms to appear alive in the fæces even though incapable of grow within the digestive tube.

The introduction of foreign organisms capable of multiplication the gastro-intestinal canal may lead to a marked alteration in t quantitative relationships of the fæcal bacteria or even to the disappe ance of certain microbic forms previously present. Thus in cholei the vibrio of this disease may occupy the intestinal canal so complete that the usual fæcal bacteria can no longer be found with the micr scope. By feeding acid-resisting lactose-fermenting bacteria, such *Bact. bulgaricum* along with considerable quantities of milk, it is possil to suppress the putrefactive anaerobes, *B. edematis* group, which pref a neutral or alkaline medium. The swallowed bacteria are manifestl therefore, of some importance in determining the character of the fæc flora, but they are, after all, usually less important in this respect tha the chemical composition of the food itself. In every case the origin intestinal flora has to be reckoned with as a most essential element.

The daily excretion* of bacteria in the feces of healthy men, i on the average, about 33 million million bacterial cells. The washe and dried substance of these bacteria amounts to about 5⅓ g. per da From one-sixth to one-fifth of the weight of the dry fæces and probab about a third of the moist fæces consists of bacterial substance. Tl nitrogen carried away by these fæcal bacteria represents a daily loss 0.5 to 1.0 g.

In addition to the bacteria, one often finds in the fæces yeasts an protozoa. Of the latter *Entamœba coli* is probably an almost constai inhabitant of the intestinal tract and its numbers are often augmente in mild chronic digestive disturbances. The flagellates, *Lambl intestinalis* and *Trichomonas intestinalis* are found less frequentl A few other protozoa occur in disease.

The physiological effects of the normal intestinal bacteria are nc fully understood. Some observers have maintained that continue life and growth would be impossible without the bacteria of the d gestive tract, ascribing to them an essential part in the nutrition c the body. The experiments of Cohendy† seem now to have disprove

* MacNeal, Latzer and Kerr, Journ. Infect. Diseases, 1909, VI, 123.
† Cohendy, Annales de l'Institut Pasteur, 1912, XXVI, 106.

ts hypothesis. There can be no doubt that the bacteria do enter imately into intestinal digestion and in some instances bring about anges beneficial to their host, such as the digestion of cellulose, ereas when furnished other food they may exert a harmful influence, for example in excessive intestinal putrefaction.

In diseased conditions of the gastro-intestinal tract one finds more less well-marked alterations in the fæcal flora. These changes clude quantitative change in the total bacterial output, change in e proportional relationships of the various normal types and finally e appearance of new or foreign types of organisms, either harmless or thogenic. In many instances there is furthermore a distinct tendency r some members of the normal intestinal flora to assume pathogenic operties and invade tissues rendered less resistant by disease.

Among the intestinal microörganisms which may assume pathonic rôles at times may be mentioned *B. coli*, *B. vulgaris*, *Ps. pyoyanea*, *B. bifidus*, *Bact. welchii*, the streptococci, micrococci and *richomonas intestinalis*. Among the definitely pathogenic forms are *. typhosus*, *Msp. comma* (*Sp. choleræ asiaticæ*), *B. paratyphosus*, *B. nteritidis*, *Bact. dysenteriæ*, *Bact. anthracis*, *Bact. pestis*, *Bact. tuberulosis*, *Entamœba dysenteriæ* (*histolytica*), *Coccidium hominis* and *amblia intestinalis*.

The technical procedures necessary for the recognition of some f these organisms in the fæces and for their isolation in pure culture re in some instances highly specific. Thus if one is searching for *B. bifidus* it is best to employ dilution cultures in tall tubes of glucose gar inoculated with fæces of a healthy nursling. The same material plated on gelatin will yield only colonies of *B. coli*. *B. welchii* is most readily isolated by pasteurizing a suspension of the fæces and introducing it into blood broth or litmus milk in a Smith fermentation tube. The cholera organism is searched for by introducing considerable quantities of fæces into flasks of pepton-salt solution and transplanting from the surface film after six hours to new flasks. On account of its very rapid multiplication in this medium the cholera germ, if present, outstrips the other fæcal bacteria. Subsequently it is necessary to apply specific agglutination tests to the spirals thus obtained in order to recognize them with certainty. The typhoid bacillus, on the other hand, is sought by inoculating media containing substances which restrain bacterial growth in general without inhibiting the growth of

B. typhosus. Broth and agar containing brilliant green are now use for this purpose.* The tubercle bacillus when present, may some times be separated by digesting the fæces in alkali or in antiformi solution, washing the residue and planting it on Petroff's medium or injecting it into guinea-pigs. *Entamœba coli* and *Entamœba dy enteriæ* should be searched for with the microscope in fresh warı fæces obtained after a dose of salts.

This brief mention of a few procedures indicates the specialize character of the microbiological technic in this field. The laborator worker will find it essential to consult the general references below an to study carefully the original papers bearing upon his field of worl

General Methods of Study

Collection of Material.—Material for microbiological study may be ol tained from the mouth, fauces or pharynx by means of a sterile cotton swab, by tl ordinary platinum loop or other instrument suitable for the special purpose in vie This material should be examined promptly, or, if this is impossible, it shoul at once be spread upon slides for subsequent microscopic study and, if cultures a to be made, it should be suspended in sterile salt solution, or better in sterile ascit fluid, and refrigerated until the proper media can be inoculated. From the stomacl fluid may be readily obtained through a stomach tube and the contents of tl duodenum or of upper portions of the small intestine may be withdrawn through tl slender duodenal tube of Einhorn. The contents of the lower part of the sma intestine and the upper part of the large intestine can be readily obtained only s surgical operations upon the intestine, at autopsies or from individuals in whom a intestinal fistula has been established. The contents of the lower part of the larg intestine are best collected by means of a special glass instrument in the case o young children. In older children and adults a natural stool or one obtaine after salts or other cathartic may be utilized.

In every instance, contamination of the material with extraneous organisms is to be strictly avoided by careful sterilization of implements and receptacles an any alteration of the specimen after collection must be reduced to the minimu by examining it promptly, although, for some purposes the use of refrigerated spec mens may be permitted.

The quantity‡ of microbic cells present may be ascertained by numerical coun of those present in an accurately measured portion of the material, or if they ar very abundant they may be physically separated out from a weighed portio by fractional sedimentation in the centrifuge, after which they are dried and weighe (method of Strasburger).

* Krumwiede, Pratt and McWilliams, Journ. Infect. Diseases, 1916, XVIII, p. 1.

† Petroff, Journ. Exp. Med., 1915, XXI, 38.

‡ For detailed directions concerning quantitative methods as applied to the study of fæca bacteria, see MacNeal, Latzer and Kerr. Journ. Infect. Diseases, 1909, VI, 123; *ibid.*, 190 VI, 571.

A preliminary classification of the recognizably different kinds of microbes ould be made by microscopic examination of film preparations stained (1) with peffler's methylene blue, (2) by Gram's method, (3) by the Ziehl-Neelsen method id (4) simply with Lugol's solution. It is best to count from 500 to 1,000 microbic lls as they are met with in successive microscopic fields and to classify them cording to form, size and structural details brought it by the different stains. Permanent records and, if ossible, permanent mounted preparations should be eserved, so that the microbes subsequently brought development in the cultures may be indentified with me of those present in the microscopic picture of ie original material.

Cultures are best made upon a quantitative basis, mploying for inoculation measured amounts of accurtely prepared dilutions of the original material. There no single culture medium or method which can be elied upon to give any approximate conception of the umerical relations of the microbes of the digestive tract. Each cultural method necessarily favors certain species resent in the mixture and allows others to develop nly poorly or not at all. Adequate information conerning the quantitative relationships is obtained only y comparing the results of the culture work with the lirect quantitative estimations and by fitting the cultural results into the original microscopic picture. A great variety of culture media and culture methods, aerobic, anaerobic and micro-aerophilic, must be employed in making even an incomplete general survey of the microbes from any portion of the digestive tract. For the detection of certain single species, on the other hand, one may sometimes rely upon a single medium, such as blood-agar for the streptococci of the mouth, Loeffler's serum for diphtheria bacilli in the pharynx or blood-broth in fermentation tube for spores of *B. welchii* in the fæces. Thus the numerous time-consuming procedures may be very much abridged and many of them may well be omitted when one wishes to ascertain merely the presence or absence of a certain single species of microbe.

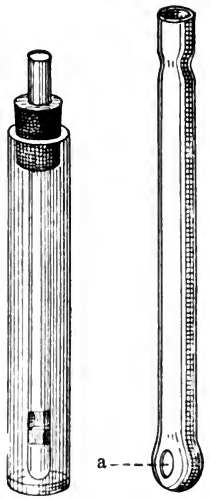

FIG. 138*A*.—Two types of instrument for obtaining fæces from infants for bacteriological examination. (*After Schmidt and Strasburger.*)

GENERAL REFERENCES

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CHAPTER VII*

THE MICROBIOLOGY OF ALCOHOL PRODUCTS

Wine

Wine may be defined shortly as the product of the alcoholic fermentation of sound, ripe grapes and the usual cellar treatment.

The classifications of wines are numerous and the varieties innumerable. They may be separated, however, into a few main groups depending on chemical composition and methods of manufacture. *Dry* wines are those in which practically all the sugar has been removed by fermentation; *sweet* wines, those in which enough sugar remains or is added to be noticeable to the taste; *fortified* wines, those that have received an addition of distilled *wine spirits;* and *sparkling* wines, those highly charged with carbon dioxide, produced by supplementary fermentation in the bottle. Each of these groups includes *white* wines made from the expressed juice of the grape, and *red* wines made from both the juice and skins of red grapes.

Grape Juice and Wine as Culture Media

Grape juice, known technically as *must*, is a sugary, acid, organic solution very favorable to the growth of yeasts and of many other fungi but unfavorable to most bacteria. Wine is of a similar composition but contains alcohol instead of sugar and is, therefore, less favorable to the growth of most microörganisms. Both liquids are of highly complex composition. Their character as culture media is indicated by the following table:

* Prepared by F. T. Bioletti.

COMPOSITION OF MUST AND DRY WINE

	Must	Wine
Specific gravity	1.0600 to 1.1090	0.9850 to 1.0000
Fermentable sugar	12.0 to 25.0 per cent	0 to 0.5 per cent
Alcohol by volume	none	8.0 to 15.0 per cent
Acidity (as tartaric)	0.5 to 1.25 per cent	0.35 to 1.0 per cent
Nitrogenous matters (soluble)	0.2 to 0.4 per cent	variable but small
Tannin	Traces	traces to 0.30 per cent
Dry extract		1.4 to 4.0 per cent
Ash	0.20 to 0.70 per cent	0.13 to 0.50 per cent

Fortified wines (sweet wines are usually fortified) usually contain enough alcohol to make them practically antiseptic to all microörganisms.

THE MICROÖRGANISMS FOUND ON GRAPES

On the surfaces of grapes, as they are brought to the cellar, may be found any of the bacteria and fungi usually carried by the air and by insects. Many of these are incapable of growing in grape must, and are, therefore, without effect on the wine.

MOLDS.—The spores of the common saprophytic molds, *Penicillium*, *Dematium*, *Aspergillus*, *Mucor*, are always present in abundance, and they find in must excellent conditions for development. *Botrytis cinerea*, a facultative parasite of the leaves and fruit of the vine, is also nearly always present in larger or smaller quantities. All these molds are harmful to the grapes and the wine. Some of them, such as *Penicillium*, may give a disagreeable, moldy taste to the wine, sufficient to spoil its commercial value. Others, such as some *Mucors* and *Aspergilli* may injure the wine but slightly except by destroying sugar and diminishing the alcohol. *Dematium pullulans* may produce a slimy condition in weak white musts and most of them may injure the brightness and flavor to some extent.

On sound ripe grapes these molds occur in comparatively small numbers and being in the spore or dormant condition they are unable to develop sufficiently to injure the wine under the conditions of proper wine making. On grapes which are injured by diseases, rain or insects,

they may be present in sufficient quantities to spoil the grapes before they are gathered. On sound grapes which are gathered and handled carelessly, they may develop sufficiently before fermentation to injure or spoil the wine.

An exception to the generally harmful effect of these molds is *Botrytis cinerea* (*Sclerotinia fuckeliana*) which under certain circumstances may have a beneficial action. When the conditions of temperature and moisture are favorable, this mold will attack the skin of the grape, facilitating evaporation of water from the pulp. This results in a concentration of the juice. The mycelium then penetrates the pulp, consuming both sugar and acid, principally the latter. The net result is an increase in the percentage of sugar and a decrease in that of acid. This, where grapes ripen with difficulty, is an advantage, as no moldy flavor is produced. Two harmful effects, however, follow: the growth of the mold results in the destruction of a certain amount of material, and a consequent loss of quantity, which is, in certain circumstances, more than counterbalanced by an increase in quality (wines of the Rhine, Sauternes); again, an *oxidase* is produced which tends to destroy the color, brightness and flavor of the wine. This can be counteracted by the judicious use of sulphurous acid.

YEASTS.—The true yeasts occur much less abundantly on grapes than the molds. Until the grapes are ripe they are practically absent, as first shown by Pasteur. Later, they gradually increase in number and on very ripe grapes often become abundant. In all cases and at all seasons, however, their numbers are much inferior to those of the molds and pseudo-yeasts. The cause of this seems to be that in the vineyard the common molds find conditions favorable to their development at nearly all seasons of the year, but yeasts only during the vintage season.

Investigations of Hansen, Wortmann and others show that yeasts exist in the soil of the vineyard at all times, but in very varying amounts. For a month or two following the vintage, a particle of soil added to a nutritive solution contains so much yeast that it acts like a leaven. For the next few months, the amount of yeast present decreases until a little before the vintage, when the soil must be carefully examined to find any yeast at all. As soon as the grapes are ripe, however, any rupture of the skin of the fruit will offer a favorable nidus for the development and increase of any yeast cells which reach it. Where

ese first cells come from has not been determined, but as there are ill a few yeast cells in the soil, they may be brought by the wind, or es and wasps may carry them from other fruits or from their hives nd nests.

The increase of the amount of yeast present on the ripe grapes is ten very rapid and seems to have (according to Wortmann) a direct lation to the abundance of wasps. These insects, passing from vine vine, crawling over the bunches to feed on the juice of ruptured erries, soon inoculate all exposed juice and pulp. New yeast cultures re thus produced, and the resulting yeast cells quickly disseminated ver the skins and other surfaces visited.

The more unsound or broken grapes present, and the more honey-ew or dust adhering to the skin, the larger the amount of yeast will e. The same is true, however, also of molds and other organisms.

In the older wine-making districts, much of the yeast present on the rapes will consist of the true wine yeast, *S. ellipsoideus*. The race or ariety of this yeast will differ, however, in different districts. Usually everal varieties will be found in each district. The idea prevalent at ne time, that each variety of grapes has its own variety of yeast seems o have been disproved, though there seems to be some basis for the dea that grapes differing very much in composition, varying in acidity nd tannin contents, may vary also in the kind of yeast present. Several varieties of *S. ellipsoideus* may occur on the same grapes. In new grape-growing districts, where wine has never been made, *S. ellipsoideus* may be completely absent.

Besides the true wine yeast, other yeasts usually occur. The commonest forms are cylindrical cells grouped as *S. pasteurianus*. These forms are particularly abundant in the newer districts where they may take a notable part in the fermentation. Their presence in large numbers is always undesirable and results in inferior wine. Many other yeasts may occur occasionally and are all more or less harmful. Some have been noted as producing sliminess in the wine. Many of these yeasts produce little or no alcohol and will grow only in the presence of oxygen.

Pseudo-yeasts.—Yeast-like organisms producing no endospores always occur on grapes. Their annual life-cycle and distribution are similar to those of the true yeasts, but some of them are much more abundant than the latter. They live at the expense of the food

materials of the must and when allowed to develop cause cloudiness and various defects in the wine.

The most important and abundant is the apiculate yeast, *S. apiculatus*. According to Lindner this is a true yeast, producing endospores. The cells of this organism are much smaller than those of *S. ellipsoideus* and very distinct in form. In pure culture these cells show various forms, ranging from ellipsoidal to pear-shaped (apiculate at one end) and lemon-shaped (apiculate at both ends). These forms represent simply stages of development. The apiculations are the first stage in the formation of daughter cells, the ellipsoidal cells, the newly separated daughter cells, which later produce apiculations and new cells in turn.

Many varieties of this yeast occur, as in the case of *S. ellipsoideus*. They are widely distributed in nature, occurring on most fruits, and are particularly abundant on acid fruits such as grapes. Apiculate yeast appears on the partially ripe grapes before the true wine yeast and even on ripe grapes is more abundant than the latter. The rate of multiplication of this yeast is very rapid under favoring conditions and much exceeds that of wine yeast. The first part of the fermentation, especially at the beginning of the vintage and with acid grapes, is, therefore, often almost entirely the work of the apiculate yeast.

The amount of alcohol produced by this yeast is about 4 per cent, varying with the variety from 2 to 6 per cent. When the fermentation has produced this amount of alcohol the activity of the yeast slackens and finally stops, allowing the more resistant ellipsoideus to multiply and finish the destruction of the sugar. The growth of *S. apiculatus*, however, has a deterring effect on that of the true wine yeast so that where much of the former has been present during the first stages of fermentation the latter often fails to eliminate all the sugar during the last stages.

When the apiculate yeast has had a large part in the fermentation, the wines are apt to retain some unfermented sugar and are open to the attacks of disease-producing organisms. Their taste and color are defective, often suggestive of cider, and they are difficult to clarify. This yeast attacks the fixed acids of the must, the amount of which is, therefore, diminished in the wine, while on the other hand the volatile acids are increased.

Many other yeast-like organisms may occur on grapes, but, under

dinary conditions, fail to develop sufficiently in competition with aiculatus to have any appreciable effect on the wine. Most of them ae small round cells, classed usually as *Torulæ*. They destroy the sgar but produce little or no alcohol.

A group of similar forms, known collectively as *Mycoderma vini*, ccurs constantly on the grapes. These, being strongly aerobic, do not velop in the fermenting vat, but under favoring conditions may be rmful to the fermented wine.

Bacteria of many kinds occur on grapes as on all surfaces exposed the air. Most of these are unable to develop in solutions so acid as ape juice or wine. Of the acid-resisting kinds, a number may cause rious defects and even completely destroy the wine. These, the *lisease-producing bacteria*" of wine, are mostly anaerobic and can evelop only after the grapes are crushed and the oxygen of the must hausted by other organisms. Practically all grape must contains me of these bacteria, which, unless the work of the wine maker is roperly done, will seriously interfere with the work of the yeast, thus using injury to the wine. The only bacteria which may injure the rapes before crushing are the aerobic, acetic bacteria, which may evelop on injured or carelessly handled grapes sufficiently to interfere ith fermentation and seriously impair the quality of the wine.

The Microörganisms Found in Wine

Wine microörganisms may be conveniently divided into two groups: nose which grow only in the presence of notable supplies of free xygen, and those which require, or grow better in, the absence of free xygen.

Aerobic Organisms. *Mycodermæ*.—If a normal wine, especially ne strong in alcohol, is left with its surface exposed to the air, it will sually, in a few days, be covered with a whitish film, thin and smooth t first but gradually becoming thicker and finally rough and plicate. This is what is known to wine-makers as "*wine flowers*." This film consists of yeast-like cells, somewhat longer and more cylindrical than *S. llipsoideus*, reproducing by budding and forming large aggregations.

Pure cultures show that there are many varieties of this organism differing in the color and texture of the film, in the cloudiness of the iquid and in the character of the deposit. They are called collectively

Mycoderma vini, though one form which has been found to produce endospores has been called *S. anomalus*.

These organisms are strongly aerobic and can develop only on the surface in full contact with the air. They are a serious enemy to the wine, rendering it insipid and cloudy. They attack the extract, fixed acids, and alcohol, producing at first volatile acids and finally causing complete combustion of the organic matters to carbon dioxide and water, destroying the wine completely.

Acetic Bacteria.—The film formed on wines exposed to the air, especially on those of low alcoholic content, will often differ from that due to *Mycoderma vini*. It will be thinner, smoother and consist of bacteria. These are the vinegar bacteria described on page 539. They grow not only on the wine at the expense of the alcohol, but on crushed grapes and must at the expense of the sugar, producing acetic acid in both cases.

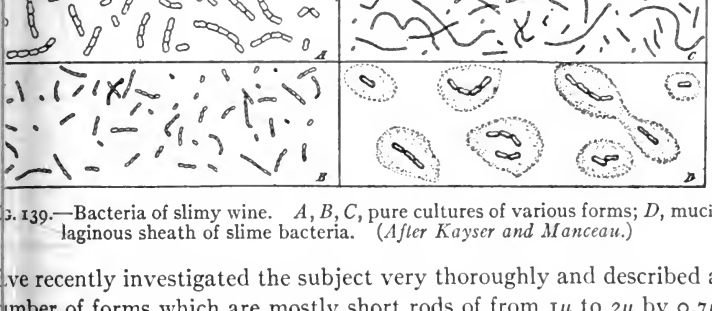
Acetic acid in small amounts is produced by the yeast and is a normal constituent of wine. Unless in excess its effect is not injurious. There may be present from 0.12 g. in 100 c.c. in light white wine to 0.14 g. in a heavy red wine without deterioration of quality. In sweet wines, even a somewhat larger amount may be present without causing injury.

Much larger amounts are injurious in two ways. When the acetic acid is perceptible to the taste, the wine is spoiled. When an abnormal amount of acetic acid is produced before or during fermentation it stops or interferes with the work of the yeast. In such cases, the wine "sticks," that is, fails to eliminate all its sugar and becomes especially open to the attacks of other bacteria.

Wines high in alcohol are less liable to acetic fermentation than weaker wines. Sound wines containing over 14 per cent by volume of alcohol are almost immune, but such wines may be spoiled during fermentation by the growth of acetic bacteria on the exposed floating "cap" of pomace or on the crushed grapes, especially at high temperatures.

Anaerobic Organisms (*Facultative and Obligate*).—Some of the worst, most frequent, and most difficult diseases and defects of wine to treat are due to organisms which develop only in the absence of oxygen. These organisms are all bacteria and appear to include a large number of forms, though, owing to difficulties of isolation and culture, the different forms have not been well studied or described.

Slime-forming Bacteria.—Musts and wines become slimy rarely t ough the action of *Dematium pullulans* (Wortmann) and wild yeast (eisner) in the presence of oxygen but more frequently through the a ion of bacteria. In most cases only young wines after fermentation a d when contained in closed casks or bottles exhibit this defect. A s ny wine has an oily appearance, pours without splashing and in e reme cases, becomes cloudy and will hang from a glass rod in strings. In such wines, the microscope reveals large numbers of almost s herical or more or less elongated bacteria in long chains. Some ob- s vers have noticed a diplococcus and a sarcina. Kayser and Manceau



G. 139.—Bacteria of slimy wine. *A*, *B*, *C*, pure cultures of various forms; *D*, muci- laginous sheath of slime bacteria. (*After Kayser and Manceau.*)

ve recently investigated the subject very thoroughly and described a mber of forms which are mostly short rods of from 1μ to 2μ by 0.7μ 1.2μ. One large form, 3μ to $4\mu \times 1.6\mu$ to 1.7μ was also noted. ey all form chains, usually of considerable length. They all produce abundant slimy sheath and stain easily with carbol-fuchsin and other iline dyes and are Gram-positive (Fig. 139).

These bacteria attack the sugar but neither the glycerin nor the cohol and produce mannit, carbon dioxide, lactic and acetic acids and hyl alcohol. The disease is usually not serious and disappears under e ordinary cellar treatment. Alcohol above 13 per cent, free tartaric id, tannin and sulphurous acid in small amounts prevent their owth.

Propionic and Lactic Bacteria.—The most serious and perhaps e commonest disease of wines is characterized by persistent cloudi- ess, disagreeable odors and flavors, increase of volatile acid and jury to the color or its complete destruction. Wines affected are

characterized commonly as *mousey*, *lactic* or *turned* wines (Pousse and Tourne of the French).

The disease is due to bacteria. Enormous numbers are readily revealed by the microscope in badly affected wines. There seem to be several or many closely related forms, all short rod-shaped, isolated in the first stages of the disease, but later forming chains or filaments of various lengths. The most noticeable change caused in the composition of the wine is the decrease of fixed and increase of volatile acidity. The tartaric acid and tartrates are destroyed, and carbonic, acetic, lactic, propionic and other acids formed.

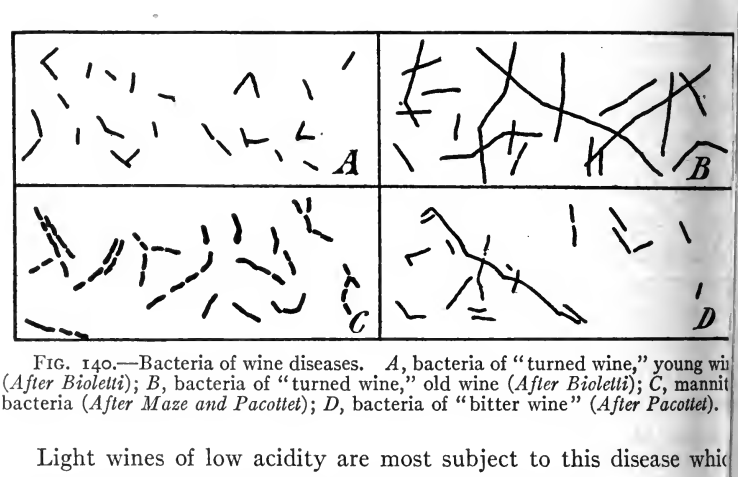


FIG. 140.—Bacteria of wine diseases. *A*, bacteria of "turned wine," young wine (*After Bioletti*); *B*, bacteria of "turned wine," old wine (*After Bioletti*); *C*, mannitic bacteria (*After Maze and Pacottet*); *D*, bacteria of "bitter wine" (*After Pacottet*).

Light wines of low acidity are most subject to this disease which may be prevented by measures which increase the acidity and alcohol by rapid and complete defecation and attenuation of the wine with the proper use of sulphurous acid, and finally by timely filtration and pasteurization. Wines noticeably affected can be used only for distilling; those badly affected are valueless.

Mannitic Bacteria.—Very sweet grapes of low acidity in hot climates are subject during fermentation to a similar trouble characterized by increase of volatile acids and a persistent cloudiness and a vapid sweet-sour taste. The disease is commonly confused with the preceding but is caused by bacteria of different forms. The form described by Gayon is a very fine short rod which does not unite in filaments. It attacks the sugar, especially the levulose, producing volatile acids and

annit. The latter may reach over 2 per cent and the former 5 per nt, giving a sweet-sour wine which is completely spoiled.

The bacteria grow abundantly only at high temperatures approach- g 40° and can be controlled by cool fermentation, increase of acidity nd proper use of sulphurous acid.

Butyric Bacteria.—In the cooler climates, wines, especially old red ines in bottles, often become bitter. This trouble is due to com- aratively large rod-shaped bacteria, first described by Pasteur. he cells remain united in angular filaments, short at first, but be- ming longer and finally thicker with age by incrustations of coloring atter.

The tannin, coloring matter, and glycerin of the wine are attacked, etic and butyric acids being formed. In small amounts the bacteria little or no harm, in larger amounts they may spoil the wine. Means hich increase the alcohol, tannin and acidity diminish the liability to e disease. Prompt attenuation and clarification and in extreme cases steurization will cure wines not too badly affected.

All the above anaerobic bacteria of wine diseases probably exist in ost wines. Which develop most, or whether any develop sufficiently injure the wine depends on conditions, chiefly the composition of the ust and the temperature at which the wine is fermented or stored. ost diseased wines show a mixed infection of several forms. Re- ntly W. V. Cruess has found bacteria in wine containing twenty r cent of alcohol. These bacteria were living and causing cloudi- ss and increasing the volatile acids.

Control of the Microörganisms

Given grapes of suitable composition, the quality of the wine epends on the work of microörganisms. The art of the wine-maker nsists almost entirely in the control of these microörganisms. His ccess in facilitating the work of the useful form (true wine yeast) d in preventing or hindering the work of injurious forms determines e quality of his product.

Before Fermentation.—On the skins of sound ripe grapes as they ng in the vineyard, the microörganisms are comparatively few and in a inactive condition. When proper methods are used they cannot ire the wine. On broken or injured grapes, the number is greater

and the forms more active. If many such grapes occur they should not be mixed with the sound grapes if the best wine is to be made.

Care should be taken to avoid unnecessary bruising of the fruit if it cannot be worked immediately. Molds, wild yeasts and acetic bacteria multiply rapidly on grapes wet with juice.

The sooner the grapes can be crushed and placed in the fermenting vat or pressed the easier it is to obtain a sound fermentation.

Cleanliness is essential. Grapes, which are gathered in moldy, vinegar-sour boxes, hauled in dirty wagons or cars, and passed through dirty crushers, conveyors and presses, may be so completely infected with injurious germs that it is impossible to obtain a good fermentation. The most injurious forms of dirt are must, grapes or wine, which have been allowed to become moldy or vinegar-sour.

Dust or soil is less injurious and, if excessive, may often be removed by sprinkling, especially is this true if the grapes are too sweet and require dilution.* Washing with antiseptics is not permissible. A weak solution of potassium metabisulphite might be used with benefit if it were not for the difficulty of regulating the amount of sulphurous acid entering the fermenting vessel.

If the grapes have to be kept for some time before crushing, they should be kept as cool as possible to delay the growth of molds. Gathering in the cool of the morning is desirable and if grapes are gathered when warm they should be left in boxes to cool off during the night whenever possible. If the grapes are cool when they reach the fermenting vat, they will neutralize a certain proportion of the heat of fermentation, and the difficulty of avoiding injuriously high temperatures is diminished.

However carefully the grapes are handled, a certain amount of dust containing germs and other injurious matters will reach the vats and presses. In the manufacture of white wines, especially, it is desirable to get rid of these matters before fermentation. This is best accomplished by settling and decantation.

As the juice runs from the press, it is pumped into a settling tank or cask. If it is cold, below 15°, and of full normal acidity, the impurities may settle in twenty to forty-eight hours. If the temperature is higher than 15° and the acidity low molds and yeasts will develop or fermentation will start and prevent settling. A slight sulphuring with the fumes of burning sulphur or with a solution of potassium metabisulphite is therefore usually necessary. The sulphuring should be as light as possible with acid musts as it tends to preserve the fixed acids. For the same reason it benefits musts of low acidity. In from twelve to twenty-four hours, the must is purged of all its gross impurities including dust, and solid particles derived from the skins and the stems and pulp of the grapes. It may be slightly cloudy or nearly clear. It can then be drawn off into clean casks and fermentation started with yeast. The microörganisms settle only in part but they are all paralyzed temporarily.

This defecation is of great value, ridding the must of substances that would affect the flavor of the wine in the heat of fermentation and eliminating the excess

* Formerly a decision of the U. S. Dept. of Agriculture forbade the use of the term "pure wine" when water in even the smallest quantities had been used. By the federal law of 1906 dilution with water up to 35 per cent. is allowed.

f protein matters that would serve as food for injurious bacteria. Centrifugal achines have been devised to hasten the process of defecation, but their work is nperfect.

Sterilization by heat has been tried for the same purpose but with indifferent ıccess. High heating caramelizes part of the sugar and oxidizes the must, thus jjuring the flavor. Discontinuous heating at lower temperatures in an atmosphere carbon dioxide is preferable but troublesome and expensive. All methods have the ffect of extracting undesirable substances from the solid matters which are heated ith the must.

Chemical sterilization is still less practicable. No substance could be used for is purpose except sulphur dioxide; this used in sufficient quantities would seriously jure the flavor of the wine. The effect would be totally different from that of the nall quantities used in defecation.

All the methods discussed have for their object the diminution or elimination of icroörganisms of all kinds. With the injurious forms the true yeast is also re-oved. The more perfect these methods, the more necessary it is to add wine east. Without this addition, in fact, all these precautions may result in harm, for e wine yeast, being present in much smaller numbers than many of the injurious rms, may be completely removed while enough of other forms are left to spoil the ine.

A "starter" of some kind is therefore necessary with defecated must nd useful in all other cases.

A Starter.—One method of producing a starter is to gather a suitable quantity the cleanest and soundest ripe grapes in the vineyard, crush them carefully nd allow them to undergo spontaneous fermentation. Perfectly ripe grapes ould be selected and the fermentation allowed to proceed until at least 10 r cent of alcohol is produced. If imperfectly ripened grapes are used or the arter used too soon, the principal yeast present will be *S. apiculatus*. Toward e *end* of the fermentation, *S. ellipsoideus* predominates. From 4 to 12 l. (1 to gallons) of this starter should be used for each 400 l. (100 gallons) of grapes or ust to be fermented. Too much starter should not be used in hot weather or th warm grapes, otherwise it may be impossible to control the temperature.

This starter is used only for the first vat or cask. Those following are started om the first fermentations, care being taken always to use the must only from a nk at the proper stage of fermentation and to avoid all tanks that show any fect.

An improvement on a natural starter of this kind is a pure culture of tested yeast. ch yeasts are being used extensively in most wine-making regions, usually with cellent results. The methods of use would require too much space to describe here, t they are simple and such as could easily be devised by anyone with some knowl-ge of microbiological technic. They do not aim at obtaining an absolutely pure rmentation, which is unnecessary, but endeavor to have an overwhelming pro-rtion of a thoroughly tested and suitable yeast which will rapidly and perfectly tenuate the wine before the few injurious microörganisms present have time to any harm.

During Fermentation.—However carefully the injurious germs have been excluded and the good yeast increased, fermentation will not be successful unless conditions as favorable to the latter and unfavorable to the former as possible are maintained.

The temperature of the crushed grapes or expressed must is of importance. If it is below 15°, unless the weather is warm, the grapes should be warmed to 20° or 25°. Unless this is done, the molds and *S. apiculatus*, which require less heat than *S. ellipsoideus*, will develop more quickly. This is especially true when starters are not used. In the warmer and earlier districts the grapes are practically never too cold. On the other hand, unless there is great carelessness, the grapes are never too warm for the commencement of fermentation. The warmer they are, however, the more artificial cooling will be necessary later, and the sooner it will have to be applied.

Thorough crushing is necessary in the case of white wine, to facilitate the expression of the juice. For red wine, the grapes are also thoroughly crushed and the skin, pulp and juice are fermented together. Imperfectly crushed grapes ferment unevenly and incompletely; thus the growth of mold is much facilitated.

The must should be thoroughly saturated with air at the beginning of fermentation to insure the multiplication of the yeast. The aeration received in the processes of stemming, crushing and pressing is usually sufficient for this purpose. More aeration would be harmful by injuring the flavor and color of the wine by over-oxidation and promoting the growth of injurious aerobic organisms. An objection to the sterilization of must by heat is the expulsion of the air and the difficulty of replacing it in the proper amount.

The proper use of sulphurous acid in the regulation of fermentation is one of the most important and necessary but least understood part of the wine-maker's art. Only by this proper use can wholesome wine of the highest quality be produced. Improper use will injure or completely spoil the wine. Its beneficial effects are due primarily to its action on microörganisms, on enzymes and on the color of the wine.

In the small quantities properly used in winemaking, it is antiseptic in a degree varying with the amount. All microörganisms are susceptible to its action in varying degrees. Bacteria are particularly sensitive, molds and psuedo-yeasts less so while wine yeast is the most resistant of the ordinary forms found in must and wine.

The result of the use of the proper amount of sulphurous acid in crushed grape

nd must before fermentation is the almost complete suppression of bacterial action, ne discouragement of molds and pseudo-yeasts and the promotion of the growth f wine yeast which is given a clear field unhindered by the deleterious excretions f competitors.

Its action as regards enzymes is hardly less important. It would be impossible to ake the finest wines of Sauternes and the Rheingau without its use on account of ne oxidase produced by the *Botrytis cinerea* which is abundant and necessary on ne best grapes of these regions. In other regions where this mold and others ccasionally occur its use is also necessary. In hot climates it is especially useful, ot only because bacterial action is more intense in such regions but because of its ction in preserving the natural fixed acids of the grape, which are, there, nearly ways deficient. This preservation, according to Wortmann, is due to the sup- ression of acid-consuming bacteria, but experiments of Astruc tend to show that ne prevention of the action of unknown acid-destroying enzymes is in part the ause.

Its action on the color of wines is also of importance. By the action of oxygen, ne color of red wine is gradually made insoluble and precipitated, and the greenish r golden color of white wine is turned to brown. Both these actions are prevented r much diminished by the use of minute quantities of sulphurous acid.

The most commonly used source of sulphurous acid is the fumes of burning ulphur. Sulphur is burned in a cask and the must caused to take up the fumes by eing pumped into the cask through the upper bung hole. It is almost impracticable o apply sulphurous acid from this source to crushed grapes for red wine.

The method is defective in many ways. It is impossible to tell within very wide mits how much sulphur dioxide has been absorbed by the wine. Moreover, the ulphur burns incompletely and the volatilized sulphur acted upon by the yeast ay produce sulphuretted hydrogen. Other sulphur compounds are also pro- uced during the burning, to some of which the so-called sulphur taste of wine is aid to be due. Several devices have been invented to decrease these defects but one remove them completely; accordingly progressive wine-makers are adopting nore reliable sources.

An improvement is the use of potassium metabisulphite ($K_2S_2O_5$) a salt which an be obtained in the requisite purity in commerce containing 50 to 55 per cent by veight of sulphur dioxide. The amount of potash added by this salt in the doses sed, is very small, and far within the limits of variation between different wines. By the use of this salt, exact amounts of sulphur dioxide can be applied both to white and red wines. Other sulphites are not permissible.

The best source of the acid, recently brought into limited use, is the liquefied gas, which can be manufactured comparatively cheaply in great purity. By its use all he benefits of sulphurous acid are obtained and the defects eliminated.

Some grapes, owing to their composition, especially their high acidity, are very resistant to the attacks of injurious bacteria. Others, owing to their low acidity or highly nitrogenous nature, are very susceptible. The addition of tartaric or citric acid to the latter has

therefore a deterring effect on some of the most dangerous forms. It is seldom necessary, however, to modify the composition for this purpose if the other means of control are used. The addition of acid or its decrease by dilution or neutralization should be solely for the direct improvement of the taste.

The quality and character of the wine depends greatly on the temperature of fermentation. If too low, the fermentation may be unduly prolonged, the wine yeast may have difficulty in overcoming its competitors and the wine may remain inferior and cloudy. With red wine the desired color, tannin and body may not be secured. On the other hand, if the temperature is too high the results are worse. The growth of bacteria is promoted, injuring the wine by the volatile acid and displeasing flavors produced and preventing the proper action of the yeast. Such wines may remain sweet on account of the failure of the yeast to do its work and become unpleasantly acid owing to the volatile acids produced by the bacteria.

Some means of controlling the temperature is therefore always needed. Where heat is deficient it may be supplied by direct heating of the must or part of it, or by heating the cellar. Where the heat is excessive, it may be diminished by crushing only cold grapes, using small fermenting vats to promote radiation and finally by the use of cooling machines applied directly to the fermenting wine.

The best temperature for fermentation depends on the kind of wine. For light white wines, the maximum should not exceed 25°, for heavier wines 30°, while for heavy red wines where high extract and tannin are required, it may be allowed to reach 35°. Sound wines can be made at all these temperatures.

As already explained, the ordinary processes of treatment of grapes result in sufficient aeration for the multiplication of the yeast. With grapes containing little sugar, this may suffice to complete fermentation. With sweeter grapes, the fermentation usually slackens when the alcohol reaches 11 or 12 per cent by volume or sooner, unless some supplementary aeration is given. With white wine this is seldom done with the result that the time of fermentation is prolonged. With red wine, the necessary stirring of the pomace to promote color extraction or the pumping over of the must in the cooling process usually gives a large amount of aeration which is sometimes excessive. Too much aeration results in extremely rapid fermentation and consequent

ifficulty in controlling the temperature. It may also have a dele-rious effect on the color, especially if sulphur dioxide has not been sed.

In any case, the main part of the fermentation should be over in om three to five days in the case of red and in from seven to fourteen ys in the case of white wine. With heavy musts, however, there will ill remain from 0.5 to 1 or 2 per cent of sugar. With certain special nes such as Sauternes it is desirable to retain the slight sweetness due this small amount of unfermented sugar. This is accomplished by e judicious use of sulphurous acid, prompt clarification by filtration or ing and when necessary by pasteurization. The pasteurization tends remove those proteins which are coagulated by heat and which are e preferred food of bacteria.

In the case of dry wines, protection from bacteria is best obtained prompt and complete attenuation. Fermentation should not be al-ved to cease until all the sugar has disappeared. For this purpose, e, two or more aerations by pumping over are usually necessary im-diately after the end of the tumultuous fermentation. The tem-rature of the wine should not be allowed to fall sufficiently to check te action of the yeast until all the sugar has disappeared.

After Fermentation.—As soon as all the sugar has been destroyed i the case of dry wines, or the desired degree of attenuation has been tained in the case of sweet wines, all the useful work of microörgan-ms has been accomplished. The quality and safety of the wine then pend on freeing it from all organisms present and preventing the e-rance and action of all others.

As soon as bubbles of carbon dioxide cease to be given off, the yeast a other solid matters will settle to the bottom and the liquid become ar. This often occurs before the fermentation is complete. In this e the yeast should be stimulated by aeration as described above.

If the wine is dry, it should be racked (drawn off, decanted) from the sediment in clean casks. The first racking is usually done while the wine is still slightly cloudy dng the first month or six weeks to remove the more bulky sediment. If left too lo on the yeast the *autophagy* or degeneration of the latter may produce substances wch injure the brightness and flavor of the wine.

A second racking is necessary at the end of winter before the spring rise of tem-pture tends to renew the activity of the microörganisms which always remain in e wine. A well made wine at this time should be perfectly bright and all solid

matters consisting of yeast and bacteria, coagulated proteins and crystals of b tartrate should have accumulated in the sediment.

Racking should take place when possible only in settled weather, when the bar metric pressure is high. Low atmospheric pressures diminish the solubility of th carbon doxide with which the wine is saturated. Under these conditions, therefor bubbles of gas are apt to be given off, bringing up particles of sediment and renderi the wine cloudy. However long wine is kept in wooden casks, it will continue deposit sediment owing to chemical changes due to the action of oxygen which pen trates slowly through the wood. Repeated rackings are therefore necessary, c curring at least twice a year until the wine is bottled or consumed.

Abundant aeration is necessary during fermentation. A moderate supply oxygen is necessary for the proper aging of wine. Experience has shown that exact the proper amount of pure filtered air will obtain access to the wine for the latter pu pose through the wood of ordinary casks of proper size. If the casks are too small t oxidation may be too rapid, if too large the maturing of the wine may be unduly p longed. The temperature of the storage cellar is the main modifying factor. T warmer the cellar the larger the casks should be.

With sound, completely fermented wines, all aeration, other than that due to porosity of the wood, should be avoided as much as possible. This is accomplish by keeping the casks tightly bunged and completely filled. Evaporation through wood continually diminishes the volume of wine and the lack must be supplied *filling up*, at first two or three times a month and later every month or two. drier the air of the cellar, the more frequent the fillings necessary.

A light sulphuring of the clean casks into which the wine is racked is us This should be practised with great caution. Very little is needed with sound wi especially if it has been used before or during fermentation and a slight excess injure the flavor. The amount should not exceed 1.25 g. per hectoliter for re 2 g. for white wine. One-half to one-third of this is sufficient for old wines. amount can be accurately measured only when using metabisulphite or the liqu gas. The utility of the sulphur dioxide with perfectly sound wines is to dimi oxidation; with wines liable to disease, to discourage the growth of bacteria.

All manipulation of the wine should be conducted with strict attentio cleanliness. This applies especially to empty casks, pumps and hoses. T should be thoroughly cleaned immediately after use and, if of metal or absorbent material, kept perfectly dry. Utensils of wood, rubber or other po material should be preserved from bacterial or mold growth with sulphurous a

The clarification of a perfectly sound new wine may be facilitated and hast by thoroughly stirring up the yeast one or two days before racking. The yea settling carries down much of the finer suspended matter, thus effecting a r *fining*. Materials such as kaolin, pure silica sand, charcoal and filter-paper be used with the same effect. The fining, however, is never perfect and the fl of the wine is often injured. A very pure clay, known commercially as Sp clay, is used largely for clearing sweet wines where the flavor is not so deli From 75 to 125 mg. per hectoliter are used for this purpose.

The best wines are nearly always fined at least once, immediately before bot One or two finings may precede this to hasten aging, defecation and *bottle ripen*

The materials used are soluble gelatinous or albuminous substances which are pable of being coagulated and precipitated by some ingredient of the wine. The st of the commonly used substances are isinglass (ichthyocol) 2 or 3 g. per hecto- er, for white wines; the white of fresh eggs, 1 or 2 per hectoliter for red; and latin, 10 or 12 g. per hectoliter for either.

The proper quantity of the finings is dissolved in a little water diluted with wine d stirred into the cask. The tannins and acids of the wine cause a gradual coagula- n in minute particles throughout the liquid. These particles gradually coalesce, rming larger particles which include all the other floating solid matter of the wine in a net. These larger particles contracted by the alcohol then settle to the ttom, leaving the wine perfectly bright.

The coagulum consists of a combination of the gelatinous matter and the tannin. me of the latter, therefore, is removed from the wine. With astringent red wines, s may be an improvement. If there is no excess of tannin present, enough must added to combine with the finings used. With white wines which contain little no tannin, this addition is always necessary.

The amount to use varies with the quality of the finings and of the tannin and h the composition and temperature of the wine.

To precipitate commercial gelatin of good quality about an equal quantity of od tannin is necessary; isinglass properly prepared requires only from one-half to e-third this amount. Eggs require only minute quantities.

Specially prepared casein of milk is used for fining white wine. Its chief merit is t the acids of the wine alone cause its complete precipitation and no addition of nin is needed, though a little is sometimes helpful. Many other albuminous stances such as milk, blood and various proprietary preparations are also used, t they are all inferior to the three mentioned and many of them introduce foreign tters such as milk sugar and bacteria which are a source of danger to the wine.

Wines containing many disease-producing bacteria may be injured by the intro- ction of finings. The evolution of gases due to the bacterial action may prevent settling and the protein matters introduced will favor the multiplication of the ease-producing organisms. By the use of 5 to 10 g. of sulphurous acid per toliter added to the wine immediately before the addition of the gelatin, the teria may be temporarily paralyzed and the finings will then settle and remove bacteria with the other floating particles.

The bright wine should be racked from the finings very soon after the sediment settled, especially when disease-producing bacteria are numerous. This will in from ten to twenty days. If the wine is not clear in three weeks it should be ered.

Filtering is inferior to fining in producing a perfectly bright wine. It is more id, however, and is useful in clearing common wine and wines refractory to fining.

Filters of innumerable forms are used. They are of two main types. For rough aring of very cloudy wines some form of bag filter is usually employed in which the e passes through a cloth tissue. The passage at first is rapid and the filtration erfect. As the solid matter accumulates on the filtering surface, the filtration im- ves but the passage of the wine is retarded. The first wine is passed a second time

through the filter and as soon as the rate of filtration becomes too slow, the operatic must be stopped and the filtering surface renewed.

For wines containing little sediment, the filter must be *primed*. This is accor plished by putting some finings in the wine first passed through the filter. Tl priming is more effective and the output of the filter much increased if a little i fusorial earth is used with the gelatin.

For the more perfect clearing of old wines some form of pulp filter is used. The are various devices by which the wine is forced through a mass of cellulose or a bestos pulp and freed from all floating matter. Some of the best of these, careful used, remove nearly all of the bacteria present.

BEER

Beer is an alcoholic beverage made from certain cereal grains l transformation of the starch to sugar, dilution with water, and ferme tation with yeast. There is usually an addition of hops and sometim of materials containing sugar. The liquid before fermentation called *wort*.

TYPICAL COMPOSITION OF VARIOUS BEERS

	Lager	Ale	Porter	Weisbier	Temper ance bee
Water	90.40	88.30	87.30	92.00	
Alcohol (by vol.)	4.85	8.00	7.00	3.45	2.00
Extract	4.20	5.54	6.45	4.63	3.95
Sugar	1.60	1.33	1.83	1.71	1.98
Lactic acid	0.10	0.20	0.22	0.27	0.04
Ash	0.23	0.30	0.40	0.16	

RAW MATERIALS AND MICROÖRGANISMS OF BREWING

GRAINS EMPLOYED.—Barley, rice and maize are the grains most commonly us wheat, rye and oats but rarely. Cane and beet sugars and syrups sometimes fc part of the fermentable material.

YEASTS OF BEER.—The yeast used is usually one of the many for of *S. cerevisiæ*. In some spontaneously fermented beer, other yeas *Torulæ* and bacteria take part, but in ordinary beers most of these considered as disease-producing organisms and injurious.

KINDS OF BEER.—The principal varieties of beer are: *lager* beers, fermented w bottom yeasts; *ales*, fermented with top yeasts (and *Torulæ*); *porters*, similar to but dark in color owing to the use of caramelized malt; *weisbiers*, in which la bacteria are abundant; and certain local types in which bacteria produce c siderable quantities of lactic and acetic acids.

Process of Brewing

Outline.—The manufacture of beer takes place in four main stages. First, portion or all of the grain is soaked in water, allowed to germinate and then ed. This produces the *malt* which contains the enzymes necessary for the version of the starch into sugar and the disintegration of the tissues of the grain. e malt is then crushed (and usually mixed with unmalted cereals or sugar) and ted with water. This constitutes *mashing*. During this process, the starch nges to maltose and dextrins which with other matters dissolve in the water; n bacteria produce a small amount of lactic acid. The resulting solution con- utes the *wort*.

The *wort*, by the addition of yeast is fermented and changed to *beer*. The rth stage includes all manipulation of the fermented beer to prepare it for con- mption.

Malting: Production of Enzymes.—The best malt is made from rley, but for special beers may be made from wheat or other grains. *eeping* consists in soaking in water to start germination. This uires from thirty-six to seventy-two hours and causes an increase in ight of about 45 per cent. The temperature should be about 12.5°. higher, injurious molds will develop. If much lower, germination l be retarded. The water should contain little organic matter or orides, nitrates or iron salts. A little calcium sulphate is favor- e. If it contains many microörganisms it should be sterilized by ling. A very little sulphite of lime or of potassium may be used to courage molds.

During *germination* several enzymes appear, of which the most important to the wer are *diastase* which changes insoluble starch into soluble sugar, rendering it ilable for the growth of the young plant; *peptase*, which performs a similar ction as regards nitrogenous matters; and *cytase* which helps in the disintegration the cellulose. All these are necessary to prepare for the work of the yeast. en the plumule has grown to about two-thirds the length of the grain, sufficient ymes have been formed. This requires from about sixteen to twenty days.

The growth of the sprouting seed is at this point stopped by careful drying with ficial heat in a kiln. The *kilning* must be sufficiently rapid to kill the germinat- seedling quickly, but not too rapid or at too high a temperature, otherwise the ymes will be weakened or destroyed. The enzymes are more sensitive when ist, consequently the heat may be increased as drying proceeds. The process mencing at a temperature between 30° and 35° is increased gradually to 5 and 55°. In twelve to twenty-four hours, the malt should appear dry. The perature is again raised gradually for another twelve to twenty-four hours to 8-100°. The lower the temperature the lighter the color of the malt. Higher peratures, especially while the malt is moist, produce dark malt.

As soon as the kilning is finished the *radicles* are *removed* by friction and screening in special machines.

WORK OF ENZYMES AND BACTERIA.—The malt is first *crushed* by pressing between rollers to facilitate the work of the enzymes and the solvent action of the water. If *unmalted grain* is to be used as well, this must be ground and the starch made soluble by heating under pressure with three or four times its weight of water and a little malt to 80°–85° for about an hour.

The methods of *mashing* are very various. They consist in general of mixing the ground malt with warm water, bringing the mass to a temperature of 35° to 45° which is gradually raised to 60°–65° by the addition of hotter water. When the action of the enzymes commences, the heated decoction of unmalted grains is added in various ways, and the temperature controlled by additions of hot water or by heating a portion of the mash. The whole mashing process requires from two to five hours according to the methods used.

During the mashing, the starch is transformed partly into maltose and partly into dextrins. The ratio of these products will vary according to the amount of diastase present and especially according to the temperature used. At about 60° the maximum amount of maltose is produced; at higher temperature (65° to 75°) the unfermentable dextrins increase. The amount of alcohol and the amount of extract in the beer therefore depend to a great extent on the method of mashing.

During the first part of the mashing, while the temperature is about 45°, lactic bacteria develop. If their action is too intense they will render the beer unpleasantly acid. If moderate, the acidity they communicate to the wort is useful in preventing the growth of the harmful butyric bacteria which might develop.

After mashing, the wort is separated from the solid matters by drawing off, extracting the mash with hot water (*sparging*), and filtration. It is then boiled from one to eight hours according to the result desired.

Boiling sterilizes the wort, kills all bacteria and destroys all enzymes which remain. These results are obtained almost instantaneously owing to the lactic acid present. Coagulation of protein substances is also brought about, effecting a clarification of the wort. This requires one or more hours, according to the nature of the wort. It is necessary also in some cases to concentrate the wort, which is done by prolonged heating in open kettles. This may require several hours.

The *Hopping* of the wort takes place during the boiling. Sometimes the hops are added just at the end of boiling; sometimes in two or three

rtions, one of which may be at the beginning and one after boiling. ops contain an aromatic essential oil, resins and tannin. The essential l is quickly soluble and volatile. To preserve its aroma in the beer, e hops must not be boiled too long. The resins are antiseptic and lp to preserve the beer. They dissolve with more difficulty and quire longer boiling.

FERMENTATION: WORK OF YEAST.—After boiling, the wort is separated from the hop débris by straining. It is then cooled by means of frigerators consisting usually of serpentine tubes through which cold line or water runs. The hot wort runs or drips over the outside of ese tubes in contact with the air. The final temperature of the wort from 12° to 18° in top fermentation and 4° to 6° in bottom.

By this means the wort is thoroughly aerated, which is necessary for e proper work of the yeast. It also effects a partial clarification by idation which causes a precipitation of solid matters.

The fermentation takes place in two stages, the violent or tumultuus fermentation in vats and the secondary or after fermentation in sks.

During the violent fermentation, the temperature is allowed to nch a maximum of 7° to 9° with light beers, 8.5° to 10.5° with dark and ° to 20° in top fermentations. At the end of the first fermentation, e beer is cooled gradually to 3.5° or 5.0° and drawn into fermenting sks where the after-fermentation takes place.

The yeasts used in brewing vary very much. Besides the division o top and bottom yeasts, various types of each are recognized. One the chief characteristics used for this division is expressed by the pertage of the total extract fermented by the yeast. The *Saaz* type ves all the dextrins and some of the maltose untouched and produces ers light in alcohol and high in extract. The *Logos* type destroys all e maltose and much of the dextrins. The result is high alcohol and w extract. The *Frohberg* type is intermediate. These differences e probably due to differences in the amount and perhaps in the nds of enzymes.

The yeasts of spontaneously fermenting beers are of various species, *ellipsoideus*, *S. pasteurianus* and others.

To produce fermentation, yeast is taken from previous vats so long the yeast remains sufficiently uncontaminated with foreign organisms. e condition of the yeast is determined by the character of the fermen-

tation, the degree of attenuation, and by microscopic examination. In breweries where modern pure culture methods are not used, the yeast present is always of several forms or types.

In any case, after a certain number of transfers, the yeast deteriorates and finally may become thoroughly infected with bacteria. The bacteria are revealed by microscopic examination. Where pure cultures are used, contamination with foreign yeast is shown by a change in the time of spore formation. By this method a contamination of 1:200 may be discovered.

When the yeast becomes contaminated, a new start must be made with yeast from another brewery, which is uncertain or by a starter of pure yeast, which is the only reliable method.

The new start with pure yeast may be made by employing a kilogram of pure pressed yeast or a corresponding amount of liquid yeast and gradually increasing it to the desired amount by repeated small additions of sterile wort. This must be done with special precaution against contamination. Many large breweries use large pure yeast machines which produce directly sufficient yeast to start a fermenting vat.

After Treatment.—The violent fermentation requires from eight to eighteen days according to the temperature. It takes place in open vats or sometimes, in top fermentation, in barrels. When sufficiently attenuated, the beer is drawn off into large casks where the slow secondary fermentation takes place at a low temperature and the beer clears by depositing yeast and other sediment. The time required for the secondary fermentation is from six to ten weeks or, with certain types of beer, from two to four months or longer.

A certain amount of dissolved carbonic acid is necessary for the quality and keeping of the beer. This is obtained by tightly bunging the casks at a suitable stage of the secondary fermentation.

The clarification of the beer is sometimes assisted by placing a quantity of chips of beech or other tasteless wood in the casks. Top fermentation beers are often fined by the use of isinglass or animal gelatin. Low fermentation beers are usually filtered.

The beer is then ready for delivery to the consumer and is placed in barrels with precautions to retain the dissolved carbonic acid.

The clear beer may be put directly into bottles with the same precautions. Bottled beers which are to be kept for some time or which are to be shipped to a distance are pasteurized after bottling at 60° to 65°.

Diseases of Beer

Beer may show defects due to imperfections in the raw material or n the methods of manufacture. These are principally abnormal flavors nd lack of clearness.

The diseases properly so called are due to wild yeasts or to bacteria. 'he disease-producing yeasts may be derived from the starter, from the essels with which the beer comes in contact, or from the air. They evelop most commonly during the secondary fermentation or in the ottle. Some may produce a disagreeable bitterness (*S. pasteurianus*) or other unpleasant flavor (*S. fœtidus*); many produce a persistent loudiness (*S. ellipsoideus*, *S. apiculatus*, *S. exiguus*, *S. anomalus*). 'hey are to be combated by preventing contamination, by proper ttenuation and by pasteurizing.

Bacterial diseases were more common before effective methods of urifying yeasts were known.

Many forms of lactic bacteria may affect the beer, rendering it acid nd cloudy. They occur principally where the temperature is allowed o become too high and where proper care in the cleaning and steril- ation of utensils is not exercised.

Acetic bacteria may occur under the same conditions and give a aste of vinegar to the beer. They are more common in top fermented eers.

Various forms of *Sarcinæ* may cause persistent cloudiness, acid, npleasant flavors or both. This contamination may be from the air r the water and is relatively common. Their growth is most rapid t 16° to 20° and is retarded by the antiseptic properties of hops.

Several kinds of bacteria, bacilli, cocci and sarcinæ may cause the eer to become slimy or viscid and injure the flavor. This trouble is articularly common in spontaneously fermented beer.

Wort and beer, being organic solutions containing very little acidity, re favorable media for the growth of bacteria, many forms of which ay cause trouble. With modern methods of using pure yeast, leanliness and the pasteurization of bottled beer, diseases can be ontrolled.

MISCELLANEOUS ALCOHOLIC BEVERAGES

Cider and Perry

These beverages are made by the alcoholic fermentation of th juices of apples and pears respectively and come next to wine and bee in the quantities produced.

The composition of the fruit varies very much according to th variety, especially in the matters of acidity, tannin and pectic sub stances. The following analysis is that of a good cider apple:

Sugar	167.0 g. per liter.
Tannin	2.4 g. per liter.
Acidity (as sulphuric)	1.6 g. per liter.

The pectic matters vary from 2 g. to 25 g. per liter but should no be too high. Pears contain usually about the same amount of suga as apples, more tannin and much less pectic substances.

The microörganisms occurring naturally on the surface of the frui are similar to those occurring on grapes, but special forms of *Sac charomyces* are found. Pure cultures of wine yeast are used success fully in cider making where a perfectly dry cider is wanted. Where small remnant of unfermented sugar is desired, the difficulties of usin pure cultures have not yet been overcome. The wild yeasts occurrin on the fruit in large quantities usually take precedence.

Attempts to sterilize the juice by heating have not been successfu owing to the production of a persistent cloudiness. Sulphurous aci is even more effective than in grape juice in delaying or preventing th action of the microörganisms. Its use must therefore always b supplemented by a starter of pure yeast.

The principles of the control of the microörganisms, good an bad, are the same as in wine making. The same care in gatherin and keeping the fruit and in extracting and handling the juice ar necessary.

The fermentation is similar to that of wine, but the cider should b taken off the yeast sooner in order to promote clarification and th retention of a little unfermented sugar.

Cider is subject to the same bacterial alterations as wine and require the same treatment. It is more difficult to keep when made in th ordinary way and is usually consumed during the first year. It i

articularly subject to turning brown, owing to the large amount of xidase present in apple juice.

The use of sulphurous acid for preliminary defecation, pure yeast in ıe fermentation, and fining, followed by pasteurization soon after the ermentation, seem to offer the best means of improving present ıethods.

These methods were introduced into a cider-vinegar factory in alifornia by W. V. Cruess with excellent results.

Fermented Beverages of Various Fruits

Many other fruits, especially those rich in sugar and with moderate cidity, are used locally to produce alcoholic beverages. The methods f fermentation are similar to those used in wine making, but additions f sugar and water are usually made to correct defects of composition. ery often distilled alcohol is also added after fermentation to preserve ıe liquid, which is thus rendered unsuitable for an ordinary beverage.

Hydromel or Mead

An alcoholic beverage made by the fermentation of honey and water much used in eastern Europe.

Honey contains from 65 to 74 per cent of reducing sugars and from to 10 per cent of saccharose. It is diluted with water to reduce its oncentration to 22° Bal.*–24° Bal. A few yeast cells are usually resent in the honey but these are of various kinds and often unsuitble. The use of a good pure yeast is therefore advisable. As honey ontains little mineral or nitrogenous yeast food, an addition of nutritive ubstances is often necessary.

The following formulæ are recommended by Kayser and Boullanger o be used in one liter:

A.	Dicalcic phosphate	1 g.
	Ammonia	2 g.
	Bitartrate of potash	2 g.
	Magnesium sulphate	0.1 g.
B.	Maltopeptone	1.5 g.
	Bitartrate of potash	1.5 g.
	Ammonium phosphate	1.0 g.

* "Balling" refers to the degrees of the special hydrometer for determining the specific avity of saccharine solutions such as must or beer wort. Its purpose is to indicate directly ıe percentage of solids in solution at a temperature of 60°F.

The same results may be obtained by mixing from 20 to 50 per cent of grape must or apple juice with the diluted honey.

Miscellaneous Fermented Beverages

Fermented beverages of some kind are made in practically every part of the world. They are very numerous and varied but fall naturally into three groups; those made from the sweet juices of fruits or other plants in which the methods of manufacture resemble those of wine making; those made from starchy materials in which the methods resemble those of brewing; and finally those made from the milk of cows or other mammals which are discussed in Chapter IV, Div. IV.

Belonging to the first group are numerous beverages made from the juices of sugar cane, various palms, and tropical fruits. The best known of these is the Mexican Pulque made by the spontaneous fermentation of the sweet juice of the agave. Little is known about the microflora concerned, but it includes alcohol-forming organisms which produce about 6 per cent of alcohol, and bacteria which cause rapid deterioration and spoiling of the fermented product. The pulque is ready for consumption twenty-fours hours after the commencement of fermentation and cannot be kept more than a day or two.

Of the beverages produced from starchy materials the Japanese Sake Rice Beer, has been most studied. It is made from rice by the diastatic action of *Aspergillus oryzæ* and yeast fermentation. The process includes three stages. First the preparation of *koji* which consists of steamed rice on which the spores of the fungus are sown and allowed to grow at 20° until the whole mass is penetrated with mycelium. The next stage is the preparation of *moto* which is a thick liquid consisting of steamed rice, water and *koji* in which the fungus transforms the starch into sugar at 0° to 10° in a few days. Fermentation then starts spontaneously, alcohol being produced by the action of several yeasts and lactic acid by bacteria, both present accidentally. In about two weeks the moto is ready. The last stage is the principal fermentation which occurs on mixing together steamed rice, koji, moto and water. This requires two weeks. The liquid is then separated, cleared and stored. It contains a considerable amount of alcohol and and can be kept and aged like wine. Sake is said to average 18 per cent of alcohol and may reach 24 per cent, the highest alcohol content known to be produced by fermentation.

POMBE is a kind of beer made in Africa from millet seed by sprouting saccharify the starch and subsequent spontaneous fermentation in ater. It is interesting as the source of the genus *Schizosaccharomyces* hich appears to take the main part in the fermentation.

GINGER BEER is an acid, slightly alcoholic beverage made by the rmentation of a 10 to 20 per cent solution of sugar containing a few ces of ginger root. The fermentation is induced by adding small ces of the so-called ginger-beer plant which consists of *Bact. vermi-rme* and *S. pyriformis*. The bacteria form a thick gelatinous sheath d seem to live symbiotically with the yeast, each developing best i the presence of the other.

DISTILLED ALCOHOL

INTRODUCTION

USES AND SOURCES OF ALCOHOL.—Distilled alcohol is used as a verage and a medicine or for innumerable purposes in the arts and ilustries. Certain methods and sources employed for the latter pur-ses are inadmissible for the former.

In all cases, it is made by the preparation from saccharine or starchy sostances of a sugar solution suitable for the work of yeast, the rmentation of this solution, and, finally, the distillation of the alcoholic luid.

Where the raw materials are sugary, methods similar to those of ne-making, and where starchy, to those of brewing, are employed, ndified to suit the conditions of each case.

The principal potable alcohols are *brandy*, made from grapes, *rum* m sugar cane, and *whiskey* from rye or other grains. Many other srces are used and any fermented beverage will, by distillation pduce a potable spirit varying in character and quality with the srce. Industrial alcohol may be made from any substance capable of ndergoing alcoholic fermentation, the limiting factor in practice being, pncipally, the cost of the raw material per unit of alcohol.

METHODS

PREPARATION OF THE SUGAR SOLUTION.—*Saccharine Raw Materials.* —When spirits are to be made from grapes or other fruit, the juice is fer-

mented in the same way as for the corresponding beverage and then distilled. The juice, however, is diluted to 20° Bal. or less, as it is not necessary or desirable to have too much alcohol in the fermented liquid. The product is consumed directly as brandy or used to fortify sweet wines. The principal fruits used besides grapes are apples peaches, plums and cherries.

Industrial alcohol has been made from inferior or spoiled fruits and from cannery wastes, but the cost per unit of alcohol is usually high The difficulties of fermentation are great, owing to the presence of large quantities of molds and other injurious organisms, and the extraction of the juice is troublesome. A careful use of sulphites and pure yeas much simplifies the process.

Sugar cane and its products are used in several ways to produce alcohol. To limited extent the juice of the cane is fermented directly and distilled. The produc is known as *Jamaica rum.* Much larger quantities of alcohol are manufactured fror the cane-sugar molasses and appear in commerce as *rum, taffia, arrack* or *neutr spirits.*

For the making of Jamaica rum the juice is pressed from the crushed canes, an diluted with 20 per cent of vinasses (the residue of a previous distillation) to increas the acidity, and give the required flavor.

Cane molasses which contain from 50 to 60 per cent of fermentable sugar ar diluted with water or vinasses to 15°–18°Bal. and partially neutralized with lim when the acidity is excessive.

One of the principal sources of industrial alcohol is the *sugar beet.* This alcohol also used for the adulteration or imitation of potable spirits.

It may be made by the direct fermentation of the beet juice, extracted by grindin and pressing, by methodical maceration or by diffusion. Sulphuric acid is adde during extraction. This facilitates the extraction by setting free organic acids, an represses the growth of injurious microörganisms. The amount used should be suc that a minute quantity of sulphuric acid remains free.

Most beet alcohol is made from the coarser *molasses* of the sugar factories. Th molasses are diluted to 20°–30° Bal. with water, further diluted and heated wi steam and acified with sulphuric acid. The sulphuric acid neutralizes the lime whic has been used in the manufacture of the sugar, sets free the volatile acids and breal up the nitrites producing nitrogen peroxide. The liquid is then boiled for about or quarter of an hour to drive off the volatile acids and the oxides of nitrogen whic would prevent yeast fermentation. The liquid after cooling is then fermented wi yeast.

Starchy Raw Materials.—In the preparation of a fermentable solu tion from starchy materials three methods for the conversion of th starch into sugar may be used, depending respectively on the action malt, dilute mineral acids, and certain molds.

The malt used in saccharification may be made, in a manner similar to that cribed for brewing, from barley, oats, rye or maize. As the object in this case is to ıse complete conversion of the starch with as little malt as possible, the malt ould have the maximum diastatic power. For this reason, germination should be ried further than for brewing and the malt used green. Drying the malt de-oys half its diastase.

The conversion may also be accomplished by boiling one part of grain in four rts of water with hydrochloric or sulphuric acid. With the former acid, 10 per nt of the weight of the grain is used and 5 per cent with the latter. The con-rsion requires from eight to twelve hours' boiling. The starch is first converted o dextrins and then into glucose. If the boiling is too prolonged some of the glucose y be lost by conversion into caramel. The amount of acid and the time of boil-g may be much reduced by operating under 2 to 3 kg. pressure. In this case 200 ers of water are heated with 100 kg. of grain and 4 kg. of acid. Conversion occurs from 40 to 60 minutes.

The power of certain molds, especially mucors, to convert starch into gar has been utilized. *Mucor rouxii* found in Chinese yeast, *Mucor yzæ* in Ragi, and related forms have been used for this purpose. This known as the *Amylo Process*. The grain is first soaked for a few ours, then heated with twice its weight of water under a pressure of ree and a half to four atmospheres until soft and the starch rendered luble. The liquefaction of the starch is facilitated by slightly acidu-ting the water with hydrochloric acid. The mixture is then cooled 38° and inoculated with a pure culture of the *Mucor*. A current of tered air is then passed through the mass for twenty-four hours, by hich time the mycelium has permeated the mass. The temperature then reduced to 33°, pure yeast added and aeration continued for enty-four hours longer to promote the multiplication of the yeast. onversion of the starch and fermentation of the sugar then continue gether. The mucor is capable of fermenting the sugar and producing lcohol, but the yeast acts more rapidly.

The malting process is the most commonly employed. The acid process de-troys a greater part of the value of the residues of distillation and the amylo process, equiring costly special equipment and large expenditures for fuel, has not come nto general use.

The starchy substances used being usually neutral or of low acidity he sugar solutions produced would be very liable to bacterial invasion nless means of prevention were used.

In the amylo process, the sterilization of the solutions and the use of ure cultures accomplish this end. In the acid process, the minute

quantity of free mineral acid remaining in the completed solution prevents any considerable growth of bacteria. In the malting process the injurious bacteria are restrained by lactic acid produced by lactic bacteria, originating in the malt or in the yeast starter. The requisite bacteria are obtained by keeping the starter or mother yeast at 50° to 58° for a certain time. This is a favorable temperature for lactic and too high for the development of acetic or other injurious bacteria When the acidity of the solution reaches 3.5 g. to 5 g. per liter the dangerous butyric bacteria cannot develop.

Pure lactic acid may be added immediately after saccharification and the loss of sugar, due to the action of the lactic bacteria avoided, but the high cost of the pure acid prevents the practice.

Yeast being much less sensitive to the presence of certain antiseptics than bacteria it is possible to control the latter by the addition of suitable amounts of an antiseptic to the sugar solution. In certain cases moreover by gradually increasing the amount, yeast can be accustomed to concentrations of antiseptics which render the growth of bacteria impossible. In Effront's method for the preparation of distillation material, hydrofluoric acid is used. This acid is added to the mother yeast at the rate of 10 g. per hectoliter and to the sugar solution in somewhat smaller amounts. This results in the inhibition of lactic, butyric and other bacteria and an increase in the fermentative power of the trained yeast.

Fermentation.—The sugar solution properly diluted and acetified or sterilized is fermented by the addition of a mother yeast, usually taken from a previous fermentation.

The original yeast may be obtained by a spontaneous fermentation as is usual in the manufacture of rum. Such a yeast is always impure, containing various yeasts, molds and bacteria, and is therefore very variable and uncertain in its results.

In the fermentation of beet juice and beet molasses, beer yeast of the Frohberg type or special distillers yeasts are used. A starter or mother yeast is prepared for each vat or the process is made continuous by leaving one-third to one-half of the contents of a fermented vat to start a fresh addition of the sugar solution. With the latter method the yeast in time becomes weak and badly contaminated and a new start must be made with fresh yeast.

In the fermentation of solutions made from potatoes, corn or other

starchy substances, each vat is started with a mother yeast. The temperature should be kept below 30° by means of refrigeration, otherwise alcohol will be lost by the multiplication of bacteria.

By the use of pure yeast, the yield in alcohol is greater as no sugar is wasted in the production of lactic acid. The cost, however, is greater owing to the necessity of the use of more heat in sterilization.

The fermentation of sugar-cane molasses for the production of arack is brought about by the use of a mother yeast called *tapej*, prepared from *ragi* or Java yeast.

Tapej is made by mixing powdered ragi with boiled rice. In two days the rice is reduced to a semi-fluid condition and contains bacteria, molds and yeasts. The bacteria seem to have no part in the process but when too numerous are injurious. The mold *Mucor oryzæ* converts the rice starch into sugar and the yeast *S. vordemanni* produces alcohol from the sugar. The other molds present are more or less injurious.

CHAPTER VIII*

THE MANUFACTURE OF VINEGAR

Acetic Fermentation

Nature and Origin of Vinegar.—Vinegar is a condiment mad from various surgary or starchy matters by alcoholic and subsequer acetic fermentation. It should contain from 4 to 8 per cent of acet acid and natural flavoring, coloring and other matters, varying accor ing to its origin.

Acetic acid (CH_3COOH) is a monobasic organic acid, the second the fatty acid series. It is a colorless liquid with a strong suffocatir odor, crystalizing when pure at 16.7° and at lower temperatures wh diluted with from 1 to 13 per cent of water. Its specific gravity is 1.(at 0° and it boils at 118° under 760 mm. pressure, producing an inflar mable vapor. It is a solvent of many organic substances and is solub in water and alcohol in all proportions.

The metallic acetates are poisonous and are formed in most cas by simple contact of metal and acid. Certain alloys of tin resist t action of the acid.

Acetic acid is formed by the oxidation of ethyl alcohol which tak place in two stages according to the following reactions:

$$\underset{\text{Ethyl alcohol}}{C_2H_6O} + O = \underset{\text{Acetic aldehyde}}{C_2H_4O} + H_2O$$

(+ Oxygen = + Water.)

$$\underset{\text{Acetic aldehyde}}{C_2H_4O} + O = \underset{\text{Acetic acid.}}{C_2H_4O_2}$$

(+ Oxygen =)

These reactions may be brought about by chemical means, but practice they are due to the action of certain microörganisms, mair bacteria. Acetic acid is also made by the distillation of wood but t product is not suitable for consumption.

Vinegar Bacteria.—If wine, beer or a similar organic solution cc

* Prepared by F. T. Bioletti.

lining alcohol, is exposed freely to the air, it soon becomes covered th a film, the alcohol disappears, is replaced by acetic acid and the uid is converted into vinegar.

This film, the *Mycoderma aceti* of Pasteur, consists of bacteria hering by means of a glutinous sheath surrounding each cell, forming zooglea. If the film is undisturbed, the liquid remains clear until nverted into vinegar, if disturbed, portions may sink, new films form d finally a large gelatinous zoogleic mass, "the mother of vinegar," ay form in the liquid.

Sometimes, especially on liquids containing sugar and large amounts alcohol, such as sweet wines, the film formed consists, not of bacteria, t of a yeast-like fungus, *Mycoderma vini*.

Wines which have been sterilized, often remain without acetifying f a considerable time. Those containing free sulphurous acid acetify swly and with difficulty. Ordinarily at warm temperatures, exposed nes develop a bacterial film very rapidly owing to the almost nstant presence of some acetic bacteria in all wines.

Hansen was the first to show that the vinegar bacteria included more an one species. He isolated and described three species concerned h the spontaneous souring of beers. Later it was shown by A. J. own, Henneberg, and others that several other species commonly curred in vinegar factories and that many more were capable of pro- cing acetic acid in small amounts. The species which have been st thoroughly studied and which seem to occur most usually in egar factories are *Bact. aceti*, *Bact. pasteurianum*, *Bact. kützingianum*, *Bct. xylinum*, short descriptions of which follow:

Bacterium aceti (Kützing), Hansen. This speices consists of rods about 1μ or 2μ in length, somewhat constricted in the middle and lying in parallel chains in th surface film. This film is moist, smooth, veined, and forms in about twenty- fo hours at 34°. On wort gelatin, it forms gray, waxy, raised colonies which are ually round, with unbroken edges but somtimes star-shaped and consisting of se rate rod-shaped cells.

Bacterium pasteurianum, Hansen.—The cells of this species are somewhat larger th those of *aceti* and more commonly produce thread-like and swollen involution fos. The film is dry and soon becomes wrinkled. Colonies on wort gelatin are sr ller than those of *aceti*, rugose, and the cells retain their arrangement in chains. T mucilaginous sheath is stained blue with iodine-potassium iodide solution (s rated solution of KI colored brown by the addition of a few drops of an alcoholic so tion of I), in this differing from *Bact. aceti* (Fig. 141).

Bacterium kützingianum, Hansen.—The cells resemble those of *Bact. aceti*, bu are usually free or in pairs. The film resembles that of *Bact. aceti* but has a tenc ency to climb up the sides of the flask above the liquid. The colonies on wort gelati are smooth and shiny. The mucilage stains blue with the iodine solution.

Bacterium xylinum, A. J. Brown.—This species forms a thick tough, leathery filn the gelatinous substance of which stains blue with iodine and sulphuric acid.

B. acetigenus, *B. oxydans*, and *B. industrius* are motile species.

All species are strictly aerobic and grow quickly only when freel supplied with oxygen. This oxygen is necessary for the acetificatio of the alcohol. Duclaux has calculated that one centigram of th bacterial film is capable of uniting 1.3 g. of oxygen to alcohol, 130 time its own weight. The optimum temperature for most species is abou

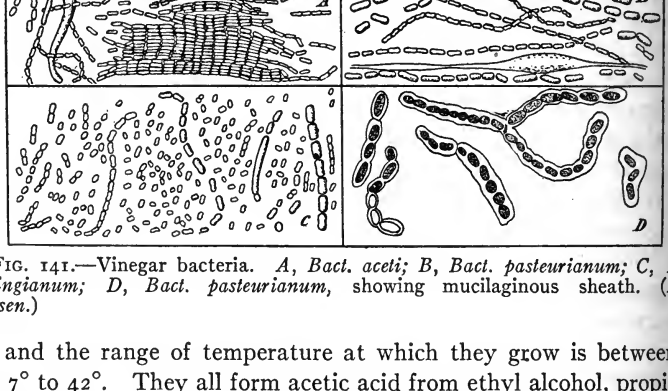


FIG. 141.—Vinegar bacteria. *A*, *Bact. aceti; B*, *Bact. pasteurianum; C*, *Bac kützingianum; D*, *Bact. pasteurianum*, showing mucilaginous sheath. (*Aft Hansen.*)

34° and the range of temperature at which they grow is between 4 and 7° to 42°. They all form acetic acid from ethyl alcohol, propioni acid from propyl alcohol and most of them gluconic acid from dextrose *B. industrius* and *B. oxydans*, according to Henneberg, can form acid from a large number of sugars and related substances, includin saccharose, maltose, starch, dextrin, glycerin and mannit.

The presence of too much alcohol prevents the growth of aceti bacteria, the limit being about 14 per cent under manufacturing con ditions. At 14 per cent and above, the film forms with difficulty, an the oxidation of the alcohol is incomplete, aldehyde and irritatin products being formed. Acetic acid in amounts above 10 to 12 pe cent is moreover antiseptic to the bacteria. Below 14 per cent o

cohol, the bacteria develop readily and produce in suitable solutions, sides acetic acid, agreeable ethers which are more abundant when e oxidation is slow. Below 1 or 2 per cent of alcohol, the bacteria tack these ethers, and finally the acetic acid itself, causing complete idation according to the equation:

$$C_2H_4O_2 + 4O = 2CO_2 + 2H_2O.$$

The addition of a new supply of alcohol, however, immediately rests this reaction. In practice the acetification should be stopped hen the alcohol has fallen to 1 or 2 per cent, otherwise there is a loss flavor and of acetic acid, which may continue until all the acid is stroyed.

The length of time during which the acetic bacteria retain their tality varies with the moisture and the temperature. In nutrient lutions, they live from one to as many as ten years; in the dry state, om three months at ordinary temperatures, to twelve months at 2°.

Processes of Manufacture

Raw Materials.—Originally vinegar was made from wine, as ilicated by the etymology of the word which means "acetified wine." later, other alcoholic beverages such as cider and beer were used for te same purpose. In these liquids, the acetic bacteria find all the neral and organic matters necessary for their development, together th alcohol in amounts favorable for acetic fermentation. At present, aarge number of materials containing alcohol, or starchy and sugary tters, which, by preliminary yeast fermentation, can be changed io alcohol are used as sources of vinegar. The most important of tse are honey, malt, and various fruit juices.

All these materials make wholesome vinegar of varying degrees of ality. Those of wine and cider are usually classed as the best, and tse of malt and honey next. The great bulk of the vinegar of comrce, however, at present is made by the acetification of distilled gin, potato and molasses alcohol. This is not vinegar strictly speakit but an imitation, consisting of a dilute solution of acetic acid with- the various flavors which are an essential part of pure vinegar. In oler to give it a semblance of the latter, it is often colored with camel and flavored with various substances.

Other imitations of vinegar sometimes appear on the market, co taining wood vinegar, or even mineral acids. These, however, are mo or less poisonous and their sale, as food, is usually forbidden by law

FERMENTATION.—If the raw material to be used is starchy sugary, it must be first changed into an alcoholic liquid containing fro 6 to 12 per cent of alcohol by volume. This is accomplished by o of the methods discussed in the preceding chapter. This alcohol fermentation must be kept rigidly distinct from the acetification and best carried out in a separate building. The yeast must finish its wo before the bacteria commence theirs. The reason for this is th yeasts are very sensitive to acetic acid and a small quantity m paralyze their activity and prevent the change of all the sugar in alcohol, with a consequent loss of strength and quality in the fin product.

The quality of the vinegar will depend on the quality of the r material from which it is made. Wine or cider spoiled by bacter fermentation, moldy casks, etc., will make inferior vinegar. exception to this may be made of so-called "pricked" wines, which a simply wines in which acetic fermentation has started spontaneous The wine or other alcoholic liquid should be perfectly clear and cle tasting and, if necessary, should be fined, filtered or pasteurized i mediately before use. It should contain no antiseptic which wo interfere with the development of the acetic bacteria. Sulphuro acid, if present in the free state, should be removed or oxidized thorough aeration.

Commerical alcohols made from corn, potatoes, beets, molasses a other products can be used. The special flavors of these alcohols, d to their origin, disappear almost completely in the vinegar. Th however, is not true of denatured alcohol or that containing met alcohol or acetone.

The alcohol must be diluted to from 10 to 12 per cent by volume, a then made suitable for the growth of acetic bacteria by the addition nutritive substances containing nitrogen and phosphates. This is complished usually by adding 10 per cent of wine, beer, malt-extra yeast decoction, or similar material to the diluted alcohol. The wa liquids from a brandy distillery may be used instead of water for dilut After resting a few days, the mixture is filtered and is then ready acetification.

Before starting the acetic fermentation, it is a usual and good practice to add about 10 per cent of good vinegar to the liquid, which is thus rendered acid and therefore less liable to alteration by injurious bacteria and other microörganisms.

All the processes of vinegar-making depend on the same principle, which is to expose the liquids prepared as above to the action of acetic bacteria with full access of atmospheric oxygen at a suitable temperature. The rapidity of the process depends on the number of active bacteria present, the nutritive value of the liquid, the temperature, and especially on the free access of oxygen.

Starters and Pure Cultures.—The 10 per cent of vinegar added to the liquid to be fermented usually contains sufficient bacteria to insure a prompt start. Where this is not the case, a starter may be prepared by exposing a suitable liquid in a shallow vessel to the air of a warm room for several days. Any liquid containing about 4 per cent of alcohol, 2 per cent of acetic acid and a moderate amount of nitrogenous matter is suitable. A decoction made by boiling 50 g. of fresh yeast in 1,000 c.c. of water, filtering and adding the proper amount of vinegar and wine or beer will serve. After thorough aeration, such a liquid in a few days becomes covered with a film of acetic bacteria. This film may be used as a starter by gently submerging in the vessel in which it is formed in the liquid to be acetified, or by removing with a clean sliver of wood which is afterward floated in the liquid.

In practice, such a starter gives a sufficiently pure fermentation of acetic bacteria. The particular species of acetic bacteria, however, is left to chance. Pure cultures of a particular selected form would in all probability improve the certainty of the production of good vinegar, but the method has not entered into general practice.

Apparatus.—Most metals of all kinds should be avoided as much as possible. The hoops of barrels and buckets may be protected by a coating of paraffin. Pumps may be of wood or of the special alloys already mentioned, or they may be so constructed that they will not come in contact with the liquids.

Domestic Method.—A cask of convenient size (40 to 200 liters) is filled as illustrated in Fig. 142.

The wine or cider to be acetified, after filtering, if necessary, is poured into the cask until it is about one-half to two-thirds full, the

object being to have as large a surface as possible for the growth of t bacterial film. Free circulation of air is insured by a 5-cm. hole each head of the cask, one near the surface of the liquid and one ne the top of the cask. These holes should be covered with varnish metal netting to prevent the entrance of flies.

The top bung hole is then closed with a cork, through which a funn passes, furnished at its lower end with a glass tube extending to with a few inches of the bottom of the cask. By means of this fun new liquid can be added without disturbing the surface film. T lower bung-hole is closed with a cork, through which passes L-shaped glass tube which serves as an indicator of level and whi also can be used to draw off the vinegar.

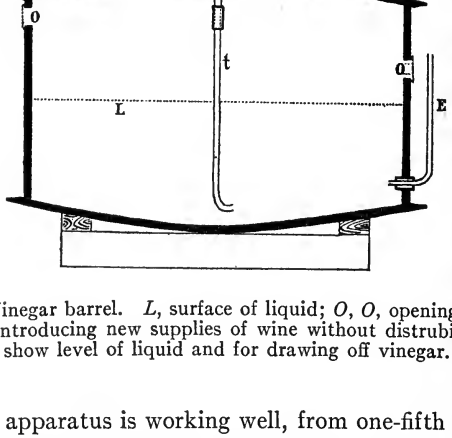


FIG. 142.—Vinegar barrel. *L*, surface of liquid; *O*, *O*, openings for entrance air; *t*, tube for introducing new supplies of wine without distrubing surface fil *E*, glass tube to show level of liquid and for drawing off vinegar. (*Original.*)

When this apparatus is working well, from one-fifth to one-quar of the contents may be taken off every three or four weeks. T depends on the temperature, which should be between 10° and 1 The vinegar drawn off is immediately replaced with wine or cider whi if added slowly, will, owing to its lower specific gravity, remain at t surface in contact with the bacterial film.

ORLEANS METHOD.—This is practically the same as the method j described with slight modifications to adapt it to large scale ope-

tions. It is the oldest commercial method and produces vinegar of the highest quality.

Barrels of about two hectoliters are usually employed, fitted essentially like that already described but with the omisson of the funnel and drawing-off tubes.

The wine is first cleared in a vinegar filter. This consists of a wooden vat filled with beech chips which have been extracted by soaking for several days in cold water. The wine remaining in contact with these chips for three or four days deposits most of its sediment.

The cask is first one-third filled with good vinegar and 10 or 15 . of the filtered wine added. The same amount of wine is added every week for four weeks by which time the cask is half full. At the end of the fifth week an amount of vinegar equal to the wine added is drawn off and the operation repeated. The vinegar is filtered as soon as it is drawn off, placed in full tightly bunged casks and kept in a cool cellar.

Pasteur Method.—Pasteur long ago pointed out the defects of the old Orleans method and suggested improvements. The main defects of the old method are that it is cumbersome, laborious, slow and costly. There is a loss of about 10 per cent of material by evaporation and the repeated additions of liquid break the bacterial film, which then sinks to the bottom, grows anaerobically and exhausts the nutrients of the solution without producing acetic acid. These submerged bacteria finally form a large gelatinous mass which interferes with the regular progress of the operations, depreciates the quality and necessitates frequent expensive cleanings of the casks. Many attempts, more or less successful, to overcome these defects in accordance with Pasteur's ideas have been made, that of Claudon is one of the best and will serve to exemplify all.

It consists essentially of a wide, shallow, covered square vat, furnished with numerous openings near the top by which the entrance of air can be facilitated and regulated. This vat is filled to the bottom of the air vents with a mixture of four parts of good new vinegar and six parts of wine which has been pasteurized at 55° and, when necessary, filtered. On top of this liquid is floated a light wooden grating which helps to support the bacterial film and prevent its breaking and submerging during the various operations. When filled, the process is started by placing a small quantity of a good bacterial film on top of

the liquid which soon becomes completely covered when the proper conditions of temperature and aeration are maintained.

Each acetifying vat is connected with a small measuring vat from which the proper amount of liquid is added every day after a corresponding amount of vinegar has been removed. These two vats constitute a unit, several of which, usually six, are united in a battery. A factory includes several of these batteries.

The batteries are fed from a large vat or reservoir, where the mixture of wine and vinegar is prepared and stored. The vinegar drawn from the batteries runs directly to filters, thence to a pasteurizer, and finally to the storage casks.

The output of these batteries is from two to five times as great per square meter of acetifying surface as that of the old method; the cost of the operation is considerably less, the loss by evaporation much reduced and the quality equal and much more under control of the manufacturer.

GERMAN METHOD.—In all the methods described, the surface of the liquid exposed to air, where alone acetification occurs, is small compared to the volume of the liquid. In order to hasten and therefore cheapen the process, various devices for increasing the surface in contact with air have been devised. The simplest of these is one sometimes employed in wine-making countries. The pressed pomace of red wine is broken up and placed loosely but uniformly in a tall narrow vat. In a few days, acetic fermentation commences in all parts of the mass. Wine is then sprinkled periodically on top and trickling down over the pomace, it is changed to vinegar by the bacterial film which encases every particle of the mass. The "quick" or German method of vinegar-making is based on this principle.

The apparatus used in this method consists of a tall cylindrical or slightly conical wooden vat provided with a perforated false head a few inches from the bottom and another, similar in structure, at the same distance from the top. The space between these two false heads is filled with long thin chips or shavings of beech wood which have been thoroughly extracted, first with water and then with good strong vinegar (Fig. 143). Various substitutes for beech chips have been used with more or less success. Rattan shavings and wastes are suitable; dried corn-cobs can be used but are not durable; wood charcoal in lumps is used successfully in the manufacture of alcohol vinegar.

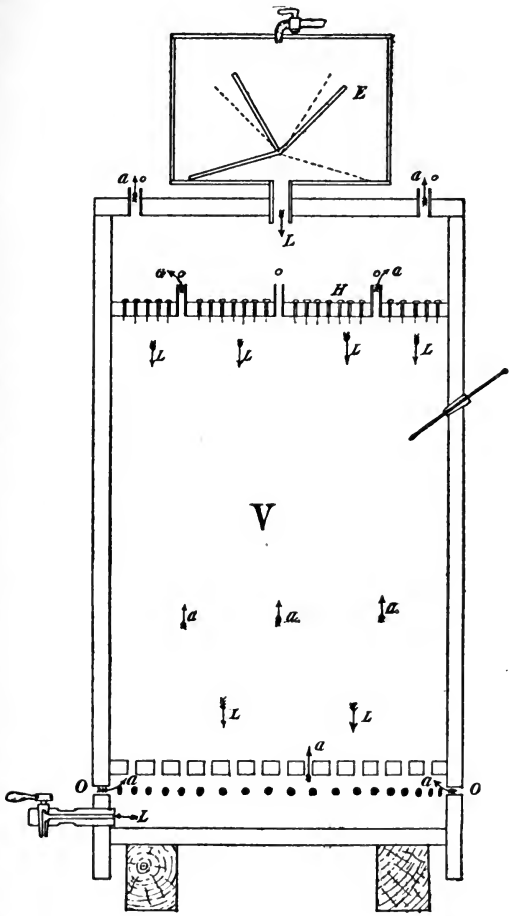


FIG. 143.—Rapid process vinegar apparatus. *V*, mass of beech chips over which alcoholic liquids run from *H*; *H*, false head with numerous small holes and threads the slow and equal distribution of the liquid; *E*, tilting trough for the intermittent supply of liquid; o_o openings for the entrance and exit of air. ↑ path of air. path of liquid. (*Original.*)

In operation, the liquid to be acetified is distributed over the to false head intermittently in small amounts. This intermittence supply is accomplished by various automatic devices. If the supply continuous, the liquid tends to run in streams or currents in certa parts of the vat and much of the acetifying surface is lost; if too rapi the bacterial film is removed from the upper part of the mass of beec chips and only the lower part is effective.

From the false head, the liquid passes through numerous small hol to the mass of beech chips, over which it trickles slowly and is acetifi by means of the bacterial film which covers them. By the time reaches the lower false head, the alcohol is in greater or less amou converted into acetic acid. Usually the liquid must pass through fro two to five times or through an equal number of vats before it is cor pletely changed into vinegar. The number of passages depends on t amount of alcohol present, the height of the acetifying column, t rapidity of the flow, the temperature, and on the perfection of t apparatus.

Oxygen is supplied by the air which, entering holes in the vat belc the lower false head, passes through numerous holes in the latte through the interstices between the chips and out through short tub fixed in the upper false head and holes in the top. The passage of a is insured by the heating of the interior due to the fermentation. can be regulated by the number and diameter of the air holes.

The temperature, which should be close to 30°, must be careful regulated. If the temperature rises too high, the loss by evaporati will be much increased; if it remains too low the acetification will retarded.

Many modifications of this method exist, having principally f their objects the more complete regulation of the temperature and a supply, the recuperation of the volatile matters, and the avoidance the need of repassing the liquid through different acetifying columns.

Rotating Barrels.—Several methods are in use which attempt combine the rapidity of the German machines with the quality of t Orleans method and which are suitable for use with wine and cid These liquids cannot be acetified conveniently by the German meth on account of the large amounts of solids and extractive matter th contain. This coats the beech chips rapidly and interferes with t perfect working of the machine.

These methods make use of a barrel filled partially or wholly with ech chips and half filled with the liquid to be acetified. By rotating e barrel at short intervals the liquid trickles repeatedly over the chips d, with proper aeration, the acetification is rapid and complete.

AFTER-TREATMENT.—Alcohol vinegars require little treatment. ey should be filtered and are usually colored slightly with caramel. ing little more than dilute solutions of acetic acid without ethers or uquet, there is no object in aging them.

Wine and cider vinegars, for the best results, require aging and reful treatment. They should be filtered and pasteurized as soon as de and stored in clean casks which are well bunged and kept con-ntly full in a cool place of even temperature. If too dark in color, ey may be decolorized with pure animal charcoal carefully extracted th acids and water.

Before using or bottling, the vinegar should be fined with isinglass (e page 523).

DISEASES

The most troublesome pest of vinegar factories is a minute nema-tle, the *Anguillula aceti* or vinegar eel. It often develops around the ges of the surface of the liquid in vinegar barrels and in the acetify-i columns and, if neglected, may cause putrefaction and spoiling of t vinegar. Frequent and thorough cleaning of all apparatus, pasteur-ition of liquids and light sulphuring of empty casks will prevent its velopment. The vinegar eels are easily killed by heating the vinegar t 50°. They may be removed by filtration or fining.

Microscopic mites are sometimes troublesome in neglected factories. ey can be reduced by the methods recommended for vinegar eels d their entrance into the barrels or acetifying columns prevented by pnting a ring of turpentine or some viscid substance around each air le.

Vinegar flies (*Drosophylla cellaris*) are often troublesome, but can be eluded by proper screening of buildings and barrels.

Bacteria other than acetic may develop in vinegar and some of m may depreciate its quality. These have been little studied but t most harmful seem to be anaerobic forms which develop in the ver parts of the liquid protected from oxygen by the screening film

of the acetic bacteria. They produce butyric acid and putrid odo and, if neglected, may completely spoil the vinegar. Sulphuring, finin and pasteurization are the remedies.

Darkening or persistent cloudiness may be caused by oxidase as wine and cider and is controlled in the same way. A similar defect ma be caused by the tannic extractive matters of new casks or contact wi iron. Aeration followed by fining will remove the cause of the troubl

CHAPTER IX*

THE MANUFACTURE OF OTHER FERMENTED PRODUCTS

Preparation and Conservation of Food Material

Compressed Yeast.—Yeast in the form of a thick paste is produced in large quantities for the use of bakers. It should be white, vigorous and rich in nitrogen.

In the *Vienna* method, a saccharine solution is made in a manner similar to that used in distilling, by saccharification with malt and acidification by lactic bacteria. Many grains, principally barley—malt, rye and corn, are used. A mixture of the three gives a solution which has the required concentration and the proper degree of viscosity to facilitate the separation of the yeast.

In the method by aeration a sweet wort is made from green malt, that is, sprouted barley. The malt is crushed, mixed with water and "mashed" for several hours at about 60° to saccharify the starch by diastatic action. The mash is then cooled to 50° and inoculated with a culture of lactic acid bacteria. When the liquid is sufficiently acidified by the action of the bacteria it is sterilized by heating to boiling. It is then filtered and cooled. This sweet wort should contain about 14 per cent of total solids.

The cooled wort is inoculated or "pitched" with a large starter of yeast and thoroughly aerated during fermentation by means of compressed air. Yeast growth and fermentation are complete in twenty-four hours or less. The yeast is separated from most of the liquid by means of a centrifugal machine and is then made drier by means of a filter press. It then forms a paste that can be molded into any desired shape or size.

The liquid or "wash" separated from the yeast is distilled and the alcohol used for the manufacture of distilled vinegar.

A small number of lactic acid bacteria in the finished product is

* Prepared by F. T. Bioletti.

considered desirable. The yeast is sometimes spoiled by bacteria causing sliminess or producing butyric acid, but this does not occur when the process is properly carried out.

BREAD.—The rising of the dough to which the lightness and porosity of bread are due is caused by the production of carbon dioxide by yeast fermentation. The yeasts are always accompanied by bacteria and the character of the bread is determined in great part by the extent of bacterial fermentation.

If we make a *dough* of flour and water and allow it to stand in a warm place it will rise slowly. Yeasts and bacteria, occurring naturally in the flour and water, are the causes. Bread is sometimes made in this way (Graham bread, salt-rising bread). The rising is more or less uncertain and the flavor and acidity of the bread very variable, owing to differences in the kind and degree of bacterial action. Many yeasts and a large number of bacteria have been isolated from the spontaneous fermentation of dough. Among the bacteria are forms producing lactic and acetic acids, others which dissolve gluten and transform starch into sugar and others which produce alcoholic fermentation with evolution of carbon dioxide.

Usually the dough is *leavened* by incorporating more or less impure yeast. Bread yeast may be prepared by allowing a culture medium composed of water, sugar, hops and potatoes with a little salt to ferment spontaneously, but the results are uncertain.

Usually, in the United States, compressed yeast is employed. In some cases the yeast from breweries is used. In most parts of Europe a leaven made from a piece of dough kept over from the last baking is preferred.

In a general way, the process consists in making a thick dough or thinner *sponge* by thoroughly mixing the flour with water, yeast or leaven and a little salt. This mixture is then allowed to stand in a warm place (20°–30°) to promote the growth and multiplication of the microörganisms. It is then kneaded, usually with more flour and put aside to rise. This kneading with fresh flour and rising may be repeated several times.

If a large quantity of a relatively pure yeast is used, the rising will be rapid, there will be little bacterial action and only one kneading is necessary. This is the method commonly employed in the bakeries in the United States. Bread made in this way is usually of fine grain, white

ar flavorless. It dries out very rapidly and is palatable only when vy fresh.

In the common household method, a smaller amount of yeast is med in a thin batter or sponge and allowed to multiply for ten to eiteen hours. This batter is then thickened to a stiff dough and alwed to rise until it doubles its volume. A second kneading is th given and a little more flour added. After rising again for a few hrs it is baked.

Bread made in this way is usually somewhat more open in texture, nso white and with more flavor. It dries less rapidly and remains patable for two or three days. The difference seems to be due prinilly to the action of bacteria on the gluten and other nitrogenous subsces in the latter case. The bacterial action, however, is not sucient to give a perceptible acidity.

Where leaven made from old dough is used, as in most parts of Sthern Europe, the part of bacteria in the fermentation is much grter.

The bread has a thicker and firmer crust, a fuller flavor and a dinct acidity which is often excessive. It holds its moisture well and kes for a week or longer.

The making of so-called French bread, as it is carried out in Paris, is a ccessful attempt to combine the good qualities of the above extres and to avoid their defects. It is based on the fact that where a lven consisting of a mixture of yeast and bacteria is used, the yeast delops more rapidly at the beginning and the bacteria at the end. Bysuccessive additions of flour and aeration by repeated kneading, sucient yeast growth is obtained to make the bread light and the baeria kept within the limits necessary for flavor and keeping qualitiewithout causing undue acidity.

YEAST AS FOOD.—Enormous quantities of yeast are produced in thvarious fermentation industries and thrown away as a waste produc after use. It has been calculated that over 60,000 tons of yeast areroduced by the breweries of Germany alone and nearly twice as mh by the distilleries. Recent tests have shown that this yeast haa highly nutritive composition and can be used as a profitable nitgenous ration for all farm animals. By careful methods, a huan food can be prepared from it which is appetizing and nearly thr times as nutritious as beef.

Large quantities have always been used as food unwittingly, eit
for animals, which consume it in certain brewery wastes of which
constitutes a large portion, or by human beings who consume it in th
bread. It has been estimated that the bread consumed in Germa
in a year contains about 147,000 tons of yeast.

Most of the yeast produced by the fermentation industries, h
ever, is wasted owing to the difficulties of transportation and c
servation. Recently methods of drying waste yeast have been
vised which overcome these difficulties and it promises to becom
valuable form of cattle food.

VEGETABLES.—Various vegetables, cabbage (sauerkraut), str
beans, cucumbers, etc., can be preserved by covering them with w
brine and allowing them to undergo spontaneous fermentation out
contact with the air.

The vegetables are cleaned, cut into pieces of convenient size, mi
with 1 to 3 per cent of salt and tightly packed in a fermentation ve
of wood, earthenware or cement. A perforated cover is placed on
and weighted down with stones. The vegetable juices are forced out
the combined action of the salt and pressure and the solid matter
duced in volume one-third or one-half.

A gaseous fermentation commences within twenty-four hours if
temperature is favorable, 18° to 20°, and continues for several we
At the end of this time the sugar in the juices has been destroyed
acids, principally lactic, produced to the extent of 0.5 to 1.0 per c
The liquid is then drawn off and replaced with 4 to 8 per cent of b
in which the vegetables will keep in good condition for a long tim
kept from the air.

The fermentation is due to a large number of microörgani
originating on the surface of the vegetables and in the water.
yeasts attack the sugar and exhaust the oxygen. The lactic bact
at the same time produce lactic acid. This is the principal fermenta
and produces the acidity to which the conservation of the mass is
Many other substances are formed by the complex fermentation,
principal products being alcohol, succinic acid, volatile acids, man
amid-bodies, carbon dioxide, hydrogen, methane and various arom
esters.

Weiss has isolated 65 different species of bacteria from sauerkr
Most of these are probably indifferent or harmless, and some harm

When the process is successful the lactic bacteria multiply rapidly from the first and quickly produce enough acidity to restrain growth of the harmful kinds, among the worst of which are the butyric bacteria.

STARCH.—Starch is prepared from potatoes, corn, wheat, flour and other amylaceous substances. The present method of separation is by chemical means. Formerly it was accomplished by a complex fermentation.

For the fermentation method, the grain is soaked in water until soft, then ground and made into a paste which is allowed to ferment spontaneously or started with a leaven taken from a previous fermentation. Alcoholic, lactic and butyric microörganisms attack the sugar while others attack the gluten and cellulose. The fermentation lasts from twelve to twenty-five days according to the temperature and the resistance of the raw materials.

During fermentation, lactic and butyric acid, hydrogen sulphide, ammonia and carbon dioxide with traces of alcohol and acetic acid are produced. The process is stopped as soon as gas ceases to be given off and before putrid fermentation sets in. The starch which is set free settles to the bottom and is separated by decantation, washing and screening.

The washed starch is then allowed to settle for three or four days in water. The sediment that is formed consists of three layers, the top consisting principally of gluten, the second of gluten and starch and the bottom of comparatively pure starch. The layers are separated and the starch extracted from the two upper layers by repeated washings on inclined planes. The starch, owing to its higher specific gravity, remains near the lower parts of these planes.

SUGAR.—In the manufacture of sugar, microörganisms have no useful part but many forms may be injurious and cause serious losses. The juices of beets and sugar cane and the saccharine liquids obtained by presses or diffusion batteries form excellent media for the multiplication of many *Saccharomyces* and bacteria. They are controlled by cleanliness, rapidity of handling, and sterilization by heat. They are injurious by destroying sugar and thereby diminishing the yield, by inverting a portion of the saccharose and rendering the crystallization difficult and by forming gelatinous masses in the liquids.

Many of them are very resistant to heat. *S. zopfii* withstands a temperature of 66° for half an hour. *Streptococcus mesenterioides* forms

chains of cocci surrounded by voluminous gelatinous sheaths whic unite in zoogleic aggregations sometimes very troublesome in sug factories. On account of its sheath it is very resistant to adver conditions. It retains its vitality after drying for three and a ha years. It is not killed by heating to 86° for five minutes and occurs i the hot liquids of the diffusion batteries.

Tobacco.—To prepare tobacco leaves for the use of the smoke they must be dried, fermented and aged. Unless all these process are properly carried out the tobacco does not possess the desired arom it contains protein substances that give a bad odor to the smoke, and usually too rich in nicotin. The processes of preparation are oft referred to as "curing," though this term is generally restricted to t preliminary drying.

The curing is more than a simple drying, as various importa changes of composition take place. When young leaves are remov from the stalk and dried they lose from 12–15 per cent of their d weight, when dried on the stalk the loss is greater; from 14–18 per cen Riper leaves lose more, up to 35 or 40 per cent. This loss is due transpiration and to translocation of substances from the leaves to t stalks and is caused by diastatic, proteolytic and other enzym The principal changes are the disappearance of starch and reduci sugar; a decrease in proteins, nicotin, pentosans and malic acid a an increase in citric acid. Ammonia is formed and the changes aft curing are more rapid with rising temperature up to about 130°F.*

After curing the dried leaves are piled in masses, moistened a allowed to undergo a fermentation which raises the temperature 50°–55°. Sometimes the leaves are then sprinkled with a soluti containing sugar, honey, various aromatic substances and sometim alcohol and passed through another fermentation.

The leaves are then tied up in bundles, partially dried, and press into boxes where another slow fermentation often takes place.

The principal chemical changes which take place in this "curin of tobacco are a considerable diminution of the nicotin, the destructi of nitrates and the production of ammonia and sometimes of buty acid.

There are three theories as to the cause of these changes. Accor

* Garner, Bacon, and Foubert, Bull. 79, U. S. Dept. of Agr., 1914.

g to Suchsland they are due to bacterial activities and by the use of ure cultures he claims to have much improved ordinary tobacco.

According to Nessler and Schlössing, the bacteria are useful only in ising the temperature of the mass which is thus made more subject the action of atmospheric oxygen. This is the immediate cause of e chemical changes.

The third theory is that of Loew who ascribes the changes to the tion of enzymes, oxidases, peroxidases and catalases existing in the aves of the tobacco.

It seems probable, according to many investigators, that the changes e due in the first place to hydrolyzing, proteolytic and oxidizing zymes, and that these diastatic transformations are supplemented the bacteria which destroy nitrates and produce ammonia; variations these various factors account for variations in the characteristics of bacco.

Preparation and Conservation of Miscellaneous Products

Indigo.—This dye was formerly made only from certain species of *digofera*, principally *I. tinctoria*. This plant contains a glucoside, *dican*, which by fermentation and oxidation yields *indigo*.

The plants are placed in water at a temperature of 25° to 35° and dergo a spontaneous alkaline fermentation which splits up the indican o a sugar (*indiglucin*) and *indigo white* which remain in the solution. his solution is then thoroughly aerated and the indigo white oxidized o *indigo blue* which is insoluble and forms a sediment. This sedi- ent is dried and constitutes the old indigo of commerce.

Many bacteria are found in the fermenting liquid, but the cause of nsformation has been shown to be a specific form, *Bacillus indigo- ous*, closely related to Friedlander's pneumonia bacillus. It is ongly aerobic and surrounded by a gelatinous envelope.

Retting.—The separation of the fibers of flax, hemp, ramie and nilar plants is brought about by a complex spontaneous fermentation. e plants are either left on the surface of grassy meadows exposed to ernate wetting and drying or immersed in water. In either case, e tissues are gradually disintegrated by microbial action, more rapidly the wet process.

The fermentation, principally bacterial, is due to many species. veral have been described as being the principal agent in the process

but it is probable that the effects are due to the united action of sever both aerobic and anaerobic.

Among the forms to which the retting has been attributed are *amylobacter* of van Tieghem, an anaerobic form which attacks the pec matters and to some extent the cellulose. *Granulobacter pectinovoru* of Beyerinck and van Delden, also anaerobic, transforms the pec matters into sugars which it decomposes, producing butyric ac Many other forms have been described and part of the work has be ascribed to *Mucor, Penicillium* and various molds.

Cultures of *Granulobacter pectinovorum* and other forms have be successfully used to hasten the process.

TANNING.—In the manufacture of leather the hides are receiv salted or dried, or more usually, fresh from the slaughter house "green hides." The green hides are thrown immediately into a containing a lime solution; the salted and dried are first soaked in wa before being placed in the lime pits. The combined action of the li and bacteria loosens the hair. The liquids in old pits contain la numbers of bacteria and are much more effective than new li solutions.

On removal from the lime pits the hair is slipped from the hides hand scrappers. Wool is removed from sheep skins after allowing th to undergo incipient putrefaction in an air-tight room.

Light hides and skins after removal of the hair are placed in a so ing pit for a few hours to remove some of the excess of lime. They a then placed in the bating wheel which is a wooden tank furnish with a large, revolving wooden wheel which keeps the hides in moti The liquor in the bating wheel contains pigeon, chicken or dog exc ments and is kept at about 40°. Rapid bacterial action takes place a the hides "fall" or become soft and most of the lime goes into soluti If left too long in the bating liquor the hides lose weight by destructi of their proteins and may finally be completely dissolved. For t reason the bating process must be closely watched and stopped as s as the hides are well softened.

Heavy hides for sole and harness leather do not go through t bating wheel, but are simply soaked and agitated in water to rem the lime. Bating would result in too much softening and loss of h substance. In some cases a dilute solution of lactic acid is used de-lime the hides.

The de-limed or bated hides go next to the "pickle," a dilute solu-
th of NaCl and H_2SO_4. Here the remaining $Ca(OH)_2$ separates as
cstals of $CaSO_4$ and is removed. The hides are "plumped" in the
pkle and increase in thickness. Formerly a fermenting mixture of
bn and water was used to plump the hides, but this has been almost
sserseded by the mineral or lactic acid pickle.

The plumped hides go progressively through a series of "tan-pits"
ctaining each a tannin solution of increasing strength, that of the
la being saturated. Tan bark is used for ordinary leather and sumach
oother light colored tannin material for light colored leather. A
laic acid fermentation occurs in the tan pits favoring osmotic action
wch tends to keep the hides plumped and thick. The tannin prob-
aly acts as a mild antiseptic, keeping down putrefaction bacteria
(V. V. Cruess.).

CHAPTER X*

MANUFACTURE OF VACCINES

Introduction

On July 1, 1902, by Act of Congress, the Secretary of the Treasu
through the Public Health Service, was placed in control of all ma
facture and sale of viruses, serums, toxins, and analogous products
human use. In order to manufacture and place such products u
the interstate market, any individual or corporation must secur
license from the Secretary of the Treasury through the Surgeon Gen
of the United States Public Health Service. All candidates, be
securing federal approval for such licenses, must allow federal inspec
the privilege of examining their laboratories, including the det
involved in the processes of manufacture. At frequent intervals
Hygienic Laboratory purchases samples of licensed products upon
open market for the purpose of subjecting these to careful examinat
If the samples of any products are found to be misrepresented a
potency or kind and amount of preservative, or if contaminating or
isms are present, the manufacturer is immediately notified to re
such products from the market.

July 1, 1913, by a similar Congressional Act, the Secretary of A
culture, through the Bureau of Animal Industry, was authorize
regulate the preparation and sale of viruses, serums, toxins
analogous products intended for use in the treatment of dome
animals.

The federal control of the manufacture of vaccines, serums, to
and other biologic products related to specific infectious diseases,
reduced to a minimum the danger formerly involved in the use of s
materials.

For one who is not a student of microbiology and preven
medicine, or not familiar with the technic involved in preparing biol

*Prepared by W. E. King.

aterials such as serums, tuberculins and vaccines, it is difficult to alize the various steps necessary in the production of a safe and active oduct. The manipulations attending the preparation of the materials quire large equipment, expensive apparatus and the services of trained boratory experts. Animals which are used in the work must be arantined and carefully inspected before being placed under treatent. The sanitary conditions of the laboratories, operation rooms d stables must be of the best.

Infection of the animal organism is due to absence of natural or quired resistance. The natural resistant forces of the animal body ay be such that insusceptibility to specific microbial invasion is esent; such a condition is called natural immunity. Acquired imunity, on the other hand, refers to a condition in which the natural sceptibility of the animal body is replaced by a temporary or rmanent resistance toward specific microbial invasions. Acquired munity may be active or passive, and may be brought about by applition of a vaccine or an antiserum. The application of smallpox ccine causes a specific reaction in the body, stimulating the developent of natural defences against smallpox virus, and is followed by a ndition of active immunity which is relatively permanent in duraon. The use of diphtheria antitoxin, which contains the antibodies pable of neutralizing the diphtheria toxin molecules, results in passive munity and affords temporary protection.

Actively Immunizing Substances (Vaccines)

Vaccines* are essentially weakened or modified viruses. Such aterials as blackleg and anthrax vaccines may be used with safety, a rule, only on animals which are free from the specific disease question, because, theoretically, if a specific vaccine were applied to patient suffering from a given infectious disease, the introduction of e attenuated organisms, or virus, would tend to augment the rulence of the infection. The action of such vaccines is preventive prophylactic, and not curative.

Attenuated Viruses.—There are several methods which may be nployed in attenuating or modifying viruses. The processes involve

* The term "vaccine" is also loosely applied to suspensions containing killed microörganns.

the treatment of viruses in such ways that they may be injected int the normal animal body without danger of producing serious symptom or lesions, while at the same time sufficient specific infectious qualitie must be present to produce mild reactions. The successful vaccin is attenuated or modified to a degree which insures both safety an activity. The following are the more important methods used t modify viruses:

Attenuation by growth at a temperature above the optimum. This i illustrated by Pasteur's method of preparing anthrax vaccine.

Attenuation by passage of the virus through some species other tha the animal for which the virus is specific. Smallpox vaccine may b regarded as a virus modified by passage through a heifer or othe animal.

Attenuation of the virus by drying at constant temperature. Th Pasteur method of prophylactic treatment for rabies is based upon thi method.

Attenuation by chemicals. The growth of certain pathogeni bacteria in the presence of weak antiseptics reduces their disease producing activities.

Other methods of modifying viruses for the purpose of active im munization:

The simultaneous method or hypodermic application of the viru together with protective serum, as in hog cholera vaccination.

The association or combination of the specific pathogenic bacteri with those of other species as illustrated by the apparent restrainin action of yeasts upon pyogenic bacteria and the antagonism whic Ps. *pyocyanea* exerts toward *Bact. anthracis.*

The filtration of liquid cultures of pathogenic organisms, such a *Bact. diphtheriæ* or *B. tetani,* and the consequent separation of th organisms from the toxin. The toxin is used to immunize animals i the production of antitoxin.

The destruction of young living cultures of specific bacteria b moist heat at a temperature slightly above their thermal death-point Heated cultures of *B. typhosus* and *Bact. pestis* are used as prophylactic against typhoid fever and bubonic plague.

There are many vaccines in practical and experimental use at th present time. Among those which are of recognized value as shown b extensive practical use and reliable clinical statistics, the followin

a the most important: smallpox vaccine, blackleg vaccine, rabies ccine, typhoid vaccine and perhaps Pasteur's anthrax vaccine. e simultaneous method, or injection of hyperimmune serum, to- her with the specific virus is used in vaccinating against hog- olera, cattle plague (Rinderpest), anthrax and foot-and-mouth ease. Asiatic cholera, bubonic plague, tuberculosis, acne, pertussis, eumonia, canine distemper, furunculosis, septicæmia hemorrhagica, norrhœa and various inflammatory processes are treated, practically d experimentally, by various methods of vaccination, either as phylactic or curative measures.

SMALLPOX VACCINE.—The first experiments relative to vaccination ainst smallpox date back to 1796. Prior to that time, the only ecific preventive method used in warding off this disease depended on the inoculation of healthy individuals with smallpox virus from mild case of the disease. The present method of vaccination util- is cowpox virus as the protective material. It has not been con- clusively determined that cowpox in cattle and smallpox in man ssess intimately related causative factors, but notwithstanding, undant evidence proves the efficacy of cowpox virus as a specific phylactic against smallpox in man.

In the practical preparation of smallpox vaccine, the virus or "seed" is first secured by removing the pulp from the vesicles which pear on infected heifers. Most laboratories which engage in this rk use a stock mixture of cowpox virus which originated from ontaneous cases of cowpox, and which is known to produce active allpox vaccine.

Great care is exercised in the selection and preparation of animals ed in making the vaccine. Heifers (calves or yearlings) are most quently used in this work, older cattle being employed in a few ropean laboratories. When first purchased these animals are ced in a detention stable where they are inspected by a qualified terinarian and carefully tested for tuberculosis. If, after several eks' quarantine, they are passed as healthy in every way, they e admitted to the vaccine laboratory after their bodies have been ubbed with soap and water and a weak antiseptic solution.

The operating room and propagating ward should be constructed th a view to thorough cleanliness. Concrete floors, enameled walls d ceilings and simple sanitary apparatus should characterize the

appointments. Floors, walls, ceilings and all equipment of these room should be carefully cleansed with disinfectant solutions at frequen intervals.

After the heifers are prepared for the work, they are inoculated wit the seed virus. The animal under treatment is placed on a speci operating table, the ventral surface of the body is shaved and cleanse and, with a sterile instrument, is scarified in parallel straight lines ov the greater portion of the abdomen and inner surface of the flank The stock virus or "seed" is inoculated in the scarified areas and th animal is released and placed in the propagating room. During th process of propagation of the vaccine all possible precautionary mea ures should be used to avoid the introduction of contaminating bacteri It is important that an attendant be constantly present, day and nigh whose duty it is to remove instantly all dirt and fæces and keep th room as clean and free from microbial contamination as possible.

At the expiration of from seven to nine days, characteristic vesicl will have developed on the inoculated areas. These are filled with thick, sticky purulent material. At this time the animal is removed f the operating table, the field of operation is washed with sterile wat and the contents of the vesicles removed with a sterile curette. Accor ing to regulations of the Federal Government all animals used in th work must be slaughtered before the vaccine is removed and lat submitted to careful autopsy. After removing the pulp, or vaccin the material is handled under aseptic precautions and mixed wit about 50 per cent glycerin, which serves as a preservative. Sma portions of the material are then inoculated into guinea-pigs for safet tests and the product is placed in the refrigerator. Under the in fluence of the glycerin extraneous microbial contamination graduall disappears. Potency tests of the vaccine are conducted by the cutan ous application of the vaccine on calves, rabbits or on slightly scarifie scrotal surfaces of guinea-pigs. In addition to the safety and potenc tests, inoculations are made into culture media which are placed unde both aerobic and anaerobic conditions to insure the absence of harmfu bacteria. For the detection of the presence of *B. tetani* the product submitted to a special test by transferring 1 c.c. into a quantity glucose beef bouillon or other special culture media, placing th culture under anaerobic conditions and incubating at body-temperatur for about ten days. After the incubation any resulting growth i

moved by filtration and the filtrate is injected into guinea-pigs. he absence of symptoms in the treated animals shows that no tetanus xin has been elaborated in the culture medium and therefore that e vaccine does not contain *B. tetani*.

After the tests are completed, the product is distributed under eptic conditions, in small, sterile, capillary tubes or upon sterile, ivory ints, sealed in sterile, glass containers properly labeled, dated and pt in the refrigerator until placed upon the market.

If kept in a cold dark place, smallpox vaccine retains its protective tivity for a considerable period. Under the influence of heat and light it rapidly deteriorates. For this reason it is difficult to ship the vaccine t tropical countries. Under suitable conditions the product should main active for a period of about one year.

Blackleg Vaccine.—The production of blackleg vaccine depends on the use of a virulent culture of *B. anthracis symptomatici*. A heifer inoculated with a small portion of the virus and rapid, acute symptms are usually produced. Death usually supervenes in about three ys. The carcass and ward are thoroughly disinfected, the body of t animal is suspended, and, after again carefully disinfecting the outse of the body, portions of the skin are removed and the muscular tsue is inspected. Those areas of the muscles which show the dark cor, gaseous formation and characteristic lesions of blackleg, are moved to the laboratory and examined microscopically for the psence of the specific organisms. After the muscle is freed from the gss connective tissue, it is suspended in strips or finely chopped, and awed to dry spontaneously. It is then ground and sterile water is aded until the mass becomes pasty or putty-like in consistency, after vich the material is placed in small shallow pans and attenuated b drying at temperature of 85° to 100° for six or seven hours. In pparing the "single vaccine" most laboratories attenuate the virus b drying at an average temperature of about 90° for six hours. In adition to the aseptic precautions observed in conducting the above pcesses, microbial contamination is practically eliminated by the dvitalization and probable death of any extraneous vegetative forms dring the attenuation process.

Blackleg vaccine (single) is tested, according to the method recommnded by the Bureau of Animal Industry, U. S. Dept. of Agriculture, afollows: A series of eight guinea pigs are injected intramuscularly

with the vaccine under test; three each with three-fourths the dose f cattle, three each with one-half dose and the remaining two with on third dose.

Temperatures of the test animals should be recorded for three su sequent days. Vaccine of proper strength is indicated when therm reactions occur in practically all the test animals together with loc reactions in some instances. None of the animals in the series shou die.

As an additional test for potency a heifer may be injected su cutaneously with one dose and a few weeks after the vaccination t animal may be exposed to the disease by receiving an injection of t virulent living organisms. If the animal remains normal the activi of the product is indicated. In order to test the vaccine in regard safety, heifers may be injected with several doses each. The absence severe disturbances shows that the material may be used witho danger.

For the purpose of eliminating possible danger from the use blackleg vaccine a "double vaccine" may be employed. This consis of two vaccines, each possessing different degrees of attentuation, whi are controlled by the degree of heat and the period of time used in atten ating the organisms in the affected muscle tissue. When the fi product, either single or double blackleg vaccine, is ready for use it usually distributed in the form of a powder, prepared threads or sm pills. The latter, first suggested by Houghton in 1898, are inject hypodermically.

Rabies Vaccine.—The successful preventive treatment for rabi or hydrophobia resulted from the brilliant researches of Paste The method devised by Pasteur in 1885, with some modifications, cc tinues to be the only practical, specific preventive treatment for rabi This treatment consists of a series of vaccinations, each vaccinati involving the application of rabies virus having a known degree attentuation. In each succeeding application of modified rabies vi the patient receives increasingly more virulent material until fina active immunity is acquired and subsequent attack from the dise is successfully resisted.

The preparation of rabies vaccine begins with the attenuation o virus having a known degree of virulence. The material may be secur from an ordinary case of "street rabies." A dog suffering from t

isease is killed and a small portion of the brain removed. The brain ssue is emulsified in sterile water or salt solution and a few drops of the aterial thus suspended in liquid, are injected subdurally into a rabbit. his may easily be accomplished by trephining the skull, after anæsthezing the animal, and with a small syringe inoculating a few drops of ie suspension just under the exposed dura mater. The inoculation of dinary rabies virus usually produces symptoms of "dumb rabies" id the death of the animal in fourteen to eighteen days. In order to crease the virulent properties of the same strain of rabid material, it transmitted from rabbit to rabbit by subdural inoculations until ie incubation period is shortened to about six days. Experience has own that when the virus has reached its maximum degree of virulence r the rabbit, the animal shows symptoms on the sixth or seventh ay after inoculation. When the virus attains this degree of virulence is called "fixed virus" and may be used in the preparation of the accine. The "fixed virus" or spinal cord of the rabbit which has ccumbed to the disease in six or seven days, is removed aseptically id placed in a special drying chamber. The cords are suspended over ustic potash and dried at a temperature of 23° for a period of from ie to ten or fifteen days.

The treatment of the patient consists in the hypodermic application of the "fixed virus" which has been attenuated by drying. The act nature of the vaccine used in the initial vaccination and the time nsumed in the series of injections depend, to some extent, upon the se in hand. Frequently, the patient is first vaccinated with a suspension of a spinal cord which has been attentuated by drying for fourteen or fifteen days. On the succeeding days of the treatment use is ade of the suspension of spinal cords, which have been less and less tenuated. The treatment usually lasts about twenty days or until ie patient has received an injection of the least attenuated "fixed rus."

It is very important, when one is bitten by a rabid animal, that the asteur treatment be begun as early as possible, in order that active mmunity may be secured before the expiration of the incubation period. many of the larger cities of the United States, for some time, laboraries have been maintained for the purpose of administering the asteur treatment. More recently, commercial laboratories have

developed methods of preparation and distribution, so that any phy sician may purchase the vaccine and administer it to his patients.

Hogyes* substituted dilutions of the "fixed virus" for the drie spinal cords. For the initial treatment, a few cubic centimeters of 1:10,000 dilution was used. In the succeeding injections graduate dilutions were employed. While the work of Hogyes has been cor firmed by other investigators, the method is not generally regarde as possessing the safety of the original Pasteur treatment.

Harris† has devised a simple method of preparing the vaccine b freezing the infected spinal cord of the rabbit with CO_2 snow, and the drying the material *in vacuo* over sulphuric acid at a temperature o 10° to 15°.

The product is kept in the refrigerator in hermetically sealed vial It is claimed that the material so prepared maintains its origin strength or infectivity several months.

Cummings'‡ method consists in the dialysis of the rabic materi in standard suspensions. Dialysis for twelve to twenty-four hou possesses the advantage of destroying the infectivity of the viru without disturbing its immunizing properties.

Dorset-Niles (Hog-Cholera) Serum.§—To prepare the materi for this process of immunization it is first necessary to secure a virulen strain of hog cholera virus. This may be obtained from any typic outbreak of the disease. A specimen of blood may be drawn, asep tically, from the carotid artery of a pig suffering from the disease, an tested for activity. Frequently a given strain of virus may not produc the acute form of hog cholera. In attempting to raise the virulence o a relatively weak virus it may be passed through a series of young pi until it uniformly produces symptoms after four to six days' incubatio and death in less than fifteen days. None except a virus having th degree of activity should be used in manufacturing the hyperimmun serum.

The virulent blood used in the process of hyperimmunizatio should be obtained from susceptible pigs weighing from 25 to 50 k (50 to 100 pounds) each. The animals to be used as the "hyperir

* Hogyes, Acad. des Sciences de Budapest, Oct. 17, 1897.

† Harris, Jour. Infect. Dis., 1912, 10, p. 369.

‡ Proceedings 15th International Congress on Hygiene and Demography, Washingto D. C., 1912.

§ U. S. Bureau of Animal Industry, Bull. No. 102.

nes" should be healthy hogs, each weighing from 100 kg. to 150 kg. (220 to 330 pounds) and possessing either natural or acquired immunity t the disease. The blood is best secured from a diseased pig by spending the animal with the head down covered with a shroud t with a disinfectant solution. The neck is shaved and disinfected. small incision is made on the median line through which a specially vised bleeding knife, properly sterilized, is introduced. The blade this knife severs the large vessels at the base of the heart and the od flows through the hollow handle into sterile containers. After e blood is obtained it is defibrinated, the serum separated from the t, and the clot discarded. The number of pigs necessary to furnish ficient virus for the hyperimmunization of one hog depends upon the ight of both the virus pigs and the immune hog.

The immune hogs may be hyperimmunized either by the "slow" "quick" method. In the former, now seldom used, the animals eive several injections at intervals of every few days, each succeeding se being increased in proportion to the weight of the animal. In the uick" method the virus is injected in one large dose, the amount being ermined by the weight of the animal. The virus may be injected ramuscularly, intraperitoneally or intravenously, the latter method w being used almost exclusively. Ten days to two weeks after the perimmune hog has received the last injection of virus, the animal eady for bleeding. When bled from the tail, the end of the appenge is severed with a sharp instrument, several hundred cubic centiters of blood are collected aseptically, defibrinated, a preservative ded and the material placed in the refrigerator. This process is peated several times, at intervals of one week to ten days, when the imal is ready for final bleeding.

By this procedure all the blood is secured from the animal according t the method described for bleeding virus pigs. The "slaughter" thod, used in many laboratories, consists of only the final bleeding, us eliminating tail bleedings. As a rule the different lots of serum presenting the different bleedings from several hyperimmune hogs a mixed and the whole subjected to test. In order to test the tency of the product eight susceptible pigs, each weighing about 23 k. (50 pounds) are inoculated subcutaneously, each with 2 c.c. of vus. Six of these pigs are simultaneously injected with graduated ses (15 to 25 c.c.) of the serum under test. If the hyperimmune

serum possesses potency the test pigs should remain in a normal co dition throughout the test, except for the presence of thermal reactio and slight clinical symptoms, while the two control pigs should sho severe symptoms in five or six days and should die in less tha fifteen days.

The practical method of treatment in the field consists in the simu taneous injection of the hyperimmune serum and virus (double trea ment), into healthy hogs for the purpose of immunization. T amount of hyperimmune serum which should be injected varies fro 30 c.c. to 90 c.c., depending upon the weight of the hog to be treate Thus, a hog weighing 34 kg. to 45 kg. (75 to 100 pounds) usually r ceives 40 c.c. of serum, together with 1 c.c. of virus. The usual do of virus for hogs above 34 kg. (75 pounds) weight is 2 c.c. For pi weighing less than 23 kg. (50 pounds) ½ c.c. of virus should injected.

ANTHRAX VACCINE.—While several methods have been used in va cinating against anthrax, probably the most important, at presen is that devised by Pasteur. This method consists in the use of cultur which have been attenuated by growth on artificial culture media temperatures above the optimum. The inoculation of such attenuat cultures into healthy animals results in active immunization.

The stock culture of *Bact. anthracis* is usually obtained from t blood of a typical case of anthrax. The culture is transferred to ag or broth and incubated. Two vaccines are prepared, the first bei less active than the second. Vaccine No. 1, is made by placing suspension in sterile, physiological salt solution or other liquid, t anthrax organisms which have been grown at a temperature of 42° f a period of fifteen to twenty days. Vaccine No. 2 consists of similarly treated culture of *Bact. anthracis* which has grown at a tem perature of 42° for ten to fifteen days. Tests of both vaccines f activity and safety are made by animal inoculations. Vaccine No should kill white mice but should not cause fatal results in guinea-pi or rabbits. Vaccine No. 2 should prove fatal for both white mice a guinea-pigs, but not for rabbits.

Healthy animals are first injected subcutaneously with about 1 c of vaccine No. 1. Several days or a few weeks after the application vaccine No. 1, the second vaccine is injected. A severe reaction a sometimes death follows the use of the vaccine. Accidents of this ki

ve resulted from careless methods employed in standardizing and ministering the vaccine. The most important objection to Pasteur's thrax vaccine is due to the danger involved in the use of the ing, attenuated anthrax organisms.

Scalvo* advocates the use of the serum from animals actively im-unized to anthrax. This method may be employed either in the rm of the immune serum alone, or the immune serum and anthrax lture simultaneously.

Eichhorn† advises the use of antianthrax serum for curative rposes, and the simultaneous treatment with antiserum and a care-lly standardized spore vaccine as a preventive. When vaccine alone to be employed Eichhorn prefers the spore vaccine rather than the dinary Pasteur vaccine.

Tuberculosis Vaccine.—Among the experimental products for e prevention of animal tuberculosis may be mentioned von Behring's ovo-vaccine." The technic involved in the preparation of this ccine is not generally known. Romer‡ describes the material as ing composed of the living tubercle organisms which are dried for a riod of thirty days in sealed glass tubes. After this process of attenua-n the organisms are injected, in carefully graduated doses, into althy calves. Field tests which have been made upon calves with vo-vaccine indicated unsatisfactory results.

In human practice various tuberculins prepared both from the uillon culture and from the cellular elements of *Bact. tuberculosis* e used as therapeutic and diagnostic agents. Products containing e cellular elements are similar in nature to bacterial vaccines.

Bacterial Vaccines (Bacterins)

Opsonins may be defined as the elements in the blood or body fluids hich are capable of modifying invading bacteria in such a way that ey become ready prey to the leucocytes. In the presence of opsonins, erefore, phagocytic activity is increased. Opsonins are apparently stinct from agglutinins, lysins, and other analogous substances, cause different degrees of heat are necessary for their destruction. oreover, a given serum may agglutinate, or may exert lytic action, thout possessing opsonic activity.

* Scalvo, Centralbl. f. Bakt., 1899, 26, p. 425..
† Eichhorn, Bull. No. 340, U. S. Dept. of Agri.
‡ Romer, Beitrage z. Exp. Therapie, 1904, 7.

Wright and Douglas first advanced the theory of opsonic action, ar suggested that the subcutaneous injection of a given species of bacteri killed by heating, caused the blood of the treated individual to exe greater opsonic activity toward the species of organisms in questio The results of the work of others proved to be confirmatory.

To prepare a bacterial vaccine, the specific organism is isolated ar after being grown for twenty-four hours or longer at a temperature 37°, it is emulsified in sterile physiological salt solution, heated approximately 60°, or killed by the use of chemical agents, standardiz as to the number of bacteria present in 1 c.c. of the emulsion, and preservative added.

If the patient and attending physician are conveniently situated respect to a laboratory, the "opsonic index" may be taken before ar during the treatment. This consists in the determination of the ave age number of the given species of bacteria ingested by the leucocyt of the patient, as compared to that which the leucocytes of norm blood are capable of destroying. It is usually found that immediate following the injection of specific bacterial vaccine there is a "negati phase" during which the leucocytes of the patient destroy a small number of bacteria. This is followed by a "positive phase," characte ized by more active phagocytosis. For practical purposes the determ nation of the opsonic index is unnecessary as the clinical reaction fc lowing the injection of a given vaccine indicates correct dosage ar progressive results of the treatment.

The use of bacterial vaccines has yielded excellent results esp cially in the curative treatment of furunculosis, acne, sycosis, puerper infection, arthritis and other affections caused by pyogenic organism and in chronic infections of the genito-urinary tract. The materi may be used in the form of "autogenous" or "stock" vaccines. A autogenous (personal) bacterial vaccine is one prepared from a cultu of the specific organism isolated from the case under treatmen Bacterial vaccines, prepared from stock cultures of the specific orga isms, may be manufactured and kept until needed for use. Some the more common stock bacterial vaccines represent the followir organisms alone or in various combinations: *Strept. pyogenes*, *S pyogenes var.* (*albus*, *aureus* and *citreus*), *M. gonorrhœa*, *Bact. pertussi M. pneumoniæ*, and *B. coli communis*.

The study of bacterial vaccines occupies a position of so muc importance in preventive medicine and therapeutics that many ne

mbinations of killed bacteria are being constantly added to the l of experimental products. Some of these have been under obvation for a considerable time and are recognized as possessing luable properties.

TYPHOID FEVER.—The typhoid bacterial vaccine of Wright* is nerally accepted as a valuable preventive against infection. For pphylactic treatment, a series of three hypodermic injections (0,000,000, 1,000,000,000 and 1,000,000,000) of killed typhoid ganisms are usually given.

The curative value of typhoid bacterial vaccine has not yet been dermined.

CANINE DISTEMPER.—Ferry,† corroborated by Torrey‡ and McGwan§ found this disease to be primarily an infection of the upper rpiratory tract due to a small motile bacillus, *B. bronchisepticus*.

Ferry and Torrey proved that suspensions of killed cultures of this ganism will immunize dogs against experimental inoculations as well against the ordinary street infection. The bacterial vaccine is being ud for prophylactic purposes in graduated doses of 200, 400 and 600 llion bacteria per c.c., given at intervals of about five days.

ASIATIC CHOLERA.—Two methods of vaccination against this dase have been proposed and statistics which relate to field tests sw positive results with both. The method of vaccination resulting fn the work of Haffkine** depends upon the use of cultures of the spillum of Asiatic cholera, attenuated by growth at temperatures alve the optimum. Vaccines of different strengths are used. Kolle*** ha proposed the use of heated (killed) cultures of the organism. Sng†† has developed a vaccine for Asiatic cholera consisting of the filates from suspensions of killed and living *Msp. comma* (*Sp. choleræ asiticæ*). This vaccine is standardized in terms of immunity units, or unit "equaling the amount of immune serum which will protect a guea-pig of 250 g. weight against the intraperitoneal inoculation of ten tiles the fatal dose of living cholera organisms."

BUBONIC PLAGUE.—Practically the same methods of procedure

* Wright, Jour. of Hyg. 2, 1902, p. 385.
† Ferry, Am. Vet. Rev., 1910, Vol. 37, p. 499. Jour. of Infec. Dis., 1911, vol. 8, p. 399.
‡ Torrey, Jour. of Med. Research, 1913, Vol. 27, 291.
§ McGowan, Jour. of Path. and Bact., 1911, Vol. 15, p. 372.
** Haffkine, Brit. Med. Jour., 1895, 2. pp. 727, 1509.
*** Kolle, Deut. med. Woch., 1897, 23. p. 4.
†† Strong, Am. Med., 1903, Vol. 6, p. 272.
Strong, Philip. Jour. Sci., 1907, Vol. 2. p. 155.

have been followed in the experimental vaccination against buboni plague as in the case of Asiatic cholera. Cultures of the plague bacillu killed by heating at a temperature of 60° for one hour, have been use with success.

Sensitized Vaccine

*Besredka has developed modified bacterial vaccines known a sensitized vaccines. In the preparation of these the living micr örganisms are brought into contact with the homologous antiseru and the mixtures allowed to stand for approximately twenty-four hou at room temperature. The organisms are then removed by centrifuga ization, washed and placed in suspension. The remaining process of manufacture are similar to those employed in the preparation ordinary bacterial vaccines.

Besredka and his associates explain the advantage of sensitize vaccines by the fact that in such preparations the microörganisms, b reason of having been in contact with homologous antiserums, a sensitized with specific amboceptors. Therefore, the sensitized orga isms are capable of immediately combining with complement, wh introduced in the blood of the patient, and prompt immunization shou follow.

Both living and killed sensitized microörganisms have been us experimentally, Besredka† having advocated the use of the former devoid of harmful properties and more certain of successful result Sensitized vaccines are still in the experimental stage, and their a vantage over the ordinary bacterial vaccines is at present a debat question.

Toxin—Antitoxin Mixture.

Babes,‡ in 1895, first suggested the use of diphtheria toxin and antitoxin mixtu as a method of immunization against diphtheria. Through the work of Park a Zingher§ and others who preceded, this method is being adopted in practi especially as a means of prophylaxis against diphtheria in schools and hospita The mixture consists of active diphtheria toxin and antidiphtheric serum in proportion of 80 per cent. of the L + dose of toxin to one unit of antitoxin.

* Besredka, Compt. Rend. de l'Acad. Sci., 1902, 134, p. 1330.
† Besredka, Bull. de l'Inst., Pasteur, 1910, 8, p. 241.
‡ Babes, Bul. Acad. de Med., Paris, 1895, 34. p. 216.
§ Park and Zingher, Jour. A.M.A. 1915, 65, p. 2214.

CHAPTER XI*

TE MANUFACTURE OF ANTISERUMS AND OTHER BIOLOGICAL PRODUCTS RELATED TO SPECIFIC INFECTIOUS DISEASES.

The principles involved in serum therapy are those of passive immunization. Therefore, the employment of an antiserum as a preventive or curative measure is an attempt to supply the patient with certain specific substances which are capable of neutralizing and destroying the specific toxic materials and pathogenic microörganisms. Presumably, the patient receives nothing in antiserums which stimulate the development of protective bodies. Active immunity does not follow as in the case of vaccine treatment. As the result of serum treatment, the patient enjoys relatively temporary protection (preventive treatment), or cessation of pathologic processes (curative treatment), because of the application of specific antisubstances. The substances contained in the serum are developed in the blood of some other species, as the horse, through repeated injections of the animal with the specific organism in question or its toxin.

Antiserums are divided into antitoxic and antimicrobial serums. An antitoxic serum is one possessing substances which, in contact with the specific toxin, unite with it, forming chemically stable and physiologically inert compounds. Under the term "antitoxic serum," in addition to antidiphtheritic and antitetanic serums, are grouped antisera for the soluble toxins of *B. botulinus* (specific meat poisoning), abrin, ricin and crotin (plant toxins), snake venom and spider toxin, and the soluble toxin of *B. anthracis symptomatici* (blackleg in cattle).

The antimicrobial serums constitute the majority of serum products. Included among these are antimeningococcic, antistreptococcic, antigonococcic, antistaphylococcic, antityphoid, antidysenteric, antirabic, antipneumococcic, antituberculosis, antiplague, anticholera, antihog cholera, antianthrax serums and serums for swine erysipelas, fowl cholera,

*Prepared by W. E. King.

white scours of calves, sheeppox, foot-and-mouth disease, canine di
temper, rinderpest and spotted fever. The action of this group
directed more especially against the specific microörganisms involve
resulting in dissolution of the cells or lysis due to lytic bodies in t
antisera (bacteriolysins).

In addition to the presence of lysins in antimicrobial serums, oth
antisubstances are known to exist, as agglutinins, bacteriotropi
(opsonins), and precipitins. The antibody content of antimicrob
serums is comparatively little understood and the clinical interpre
tion of lysins, agglutinins and precipitins is not clear.

Antitoxic Serums

Diphtheria Antitoxin.—A culture of the organism may readily
secured from the throat of a patient by transferring some of the di
theritic exudate, on a sterile cotton swab, to Loeffler's blood-ser
culture medium. After the growth of the bacteria at incubator te
perature, contaminating organisms may conveniently be eliminated
the inoculation of a guinea-pig and the isolation of the diphtheria org
isms from the tissues. A pure culture is necessary in the preparat
of the antitoxin, but any given culture should not be relied upon u
tests have been made of the final product.

To produce the diphtheria toxin with which the antitoxin horses
treated, the diphtheria organisms, in pure culture, are transferred
beef broth, contained in large flasks, and incubated at a temperature
37°. A rapid growth takes place, during which the specific toxi
elaborated by the organisms. After a period of incubation of ab
two weeks, the bouillon culture is removed from the incubator, exami
microscopically in order to make sure that contamination is not pres
a preservative is added, usually carbolic acid, trikresol, or purified
sols, and the organisms are removed from the culture by passing
liquid through a Berkefeld filter. The filtrate (diphtheria toxin
then placed in the refrigerator until used.

The horses which are used in the manufacture of antidiphther
serum, as well as for the preparation of other antiserums, must
submitted to rigid inspection before being placed on the treatm
These animals, when purchased, are placed in a detention stable
several weeks. During this time they are subjected by a quali

terinarian to a thorough physical examination and to the mallein st for glanders. Finally, only those animals which are pronounced rmal in every way are admitted to the antitoxin stables. The stables d the operating rooms with their appointments, which are designed f the antitoxin horses, should be constructed with a view to perfect nitation and cleanliness. Concrete floors, sanitary stalls, mangers, cks and apparatus, good water, free ventilation and plenty of light uld characterize the quarters.

The antitoxin horses are injected subcutaneously with the diphtheria kin. The initial dose of toxin usually consists of only a fraction of a bic centimeter, then increasingly larger doses are administered until t animals are finally able to receive 300 c.c. or more at a single treatnt. The intervals between injections and the rate of increase of sceeding doses at any given time depend upon the condition of the amal. During this treatment a constant process of antitoxin formatn is taking place in the body of the horse. In order to produce a pot t serum, the injection of the toxin should be continued throughout t course of treatment as rapidly as the resulting reactions, following eh injection of the animal, will allow.

After the completion of the initial toxin treatment, which occupies a period of from six weeks to three months, the horse is allowed a rt of about two weeks, during which time all the toxin which has been iected should be absorbed. During the remainder of the antitoxinducing period of the animal's life (approximately two years), treatnt with diphtheria toxin is continued at regular intervals. When dired, a small sample of blood serum may be secured from the horse for pliminary potency tests. Finally, the animal is bled from the jular vein, under aseptic conditions. As much blood is secured as th horse can conveniently yield, varying in quantity from 10 to 15 l. Te blood may be drawn through a sterile canula and rubber tube into t, sterile glass cylinders. After the blood has clotted the serum sarates and at the end of twenty-four to forty-eight hours, the clear, aber-colored fluid is poured from the cylinders into large, sterile glass ctainers, a preservative is added and the material is transferred to th laboratory. The serum is then passed through a Berkefeld filter.

Each lot of antidiphtheritic serum is submitted to rigid tests rtive to potency, safety and microbial contamination. In determing the potency, varying amounts of the serum under test are

mixed with the L+ dose of diphtheria toxin and injected into a seri of guinea-pigs, each weighing 250 g.* The L+ dose of toxin is the lea amount of toxin, which, when mixed with one unit of standard antitoxi (supplied by the Hygienic Laboratory) and injected into a guinea-p of 250 g. weight, is sufficient to kill the animal in four days. From th results of this test it is possible to determine the smallest amount of th antitoxin which will protect a guinea-pig of 250 g. weight, when th animal has received simultaneously the L+ dose of toxin. This min mum amount of antitoxin represents one unit. Thus, if $\frac{1}{500}$ c.c. of th given antitoxin represents the smallest amount which is capable neutralizing the L+ dose of toxin, then the antitoxin would possess potency of 500 units per c.c.

In order that the antitoxin may be tested for safety, each of sever guinea-pigs is injected subcutaneously with about 2 c.c. of the serur These animals are not released until the observer is satisfied that th serum contains no injurious properties. For the purpose of detectic of microbial contamination, relatively large amounts of the antitoxi are placed in culture media and incubated under both aerobic ar anaerobic conditions.

Diphtheria antitoxin is usually distributed in glass syringe containe ready for immediate use. After the product has been tested relati to potency, safety and microbial contamination, it is put up in steri glass cylinders. These cylinders are so constructed that accompanyir sterilized needles and pistons may be conveniently applied and the ant toxin injected hypodermically directly from the containers. Ea container must bear a label indicating the number of antitoxin uni enclosed and the date of preparation.

Finally, after the diphtheria antitoxin has been distributed in th glass cylinders, sealed and packed ready for use, sample packages a opened and examined for contamination, usually by two microbiologist The product is not approved until the independent results of these fin tests are compared, and it is assured that microbial contamination absent.

All antitoxic serums should be kept away from the light and at temperature of 10° to 15° whenever possible, as the presence of he and light causes gradual deterioration. Usually an expiration date from eighteen months to two years is applied to diphtheria antitoxi

* See Bulletin No. 21, Hygienic Laboratory, Washington, D. C.

It has been demonstrated that the antitoxic content of serum is losely associated with the globulins. Advantage is taken of this act by most laboratories in reducing the volume of antitoxin, or oncentrating the product, by precipitating the globulins with mmonium sulphate, redissolving the precipitate and dialyzing. The concentration of serum by this method increases the unit value er volume and tends to decrease the occurrence of undesirable secondry effects ("serum sickness").

TETANUS ANTITOXIN.—The processes involved in the preparation f antitetanic serum differ but little from those employed in the anufacture of diphtheria antitoxin. The pure culture of *B. tetani* inoculated into large flasks of glucose bouillon, placed under anerobic conditions and incubated at body temperature. A convenient ethod of excluding free oxygen, in the presence of which the tetanus rganisms will not multiply, consists in boiling the glucose bouillon efore the inoculation, to drive off the oxygen, then covering the quid medium by a layer of oil. These cultures are subjected to a mperature of 37° for several weeks, after which they are examined icroscopically, a preservative is added and they are passed first hrough a Berkefeld filter, and finally through a Pasteur filter. On ccount of the presence of spores and the danger attending the conmination of any materials or biological products with the tetanus acillus, it is important that great care should be exercised in the tration and preparation of the tetanus toxin. Therefore, the filtraon process is best accomplished in an isolated room which is used nly for the preparation of tetanus toxin.

Tetanus antitoxin is produced by the injection of horses with the ecific toxin and the same general methods and precautions are obrved as in the preparation of diphtheria antitoxin. The antitanic serum is tested relative to potency, safety and freedom from icrobial contamination. *The standard unit of tetanus antitoxin is garded as ten times the least quantity of antitetanic serum necessary save the life of a 300-g. guinea-pig for ninety-six hours, against the ficial dose of a standard toxin furnished by the Hygienic Laboratory the Public Health Service.

Tetanus antitoxin is put up for use in the same manner as diph-

* See U. S. Treasury Department, Public Health Reports, Vol. XXIV, No. 20, 1904.

theria antitoxin, being usually distributed in glass syringe containers. The product is used in both human and veterinary practice.

Antimicrobial Serums

In addition to diphtheria and tetanus antitoxins, certain other antiserums are rapidly attaining practical significance. At present however, no methods are in use by which any antiserums other than diphtheria and tetanus antitoxins can be accurately standardized as to potency. Nevertheless, most of the products can be submitted to rigid tests in order to determine the presence of protective qualities.

Antimeningococcic Serum.—Horses are immunized to cultures of a number of strains of *M. intracellularis var. meningitidis*, the activity of the resulting serum being determined by agglutination and complement fixation tests. Antimeningococcic serum is used in the active treatment of cerebrospinal meningitis and is administered by lumbar puncture. The dose depends principally upon the age of the patient and the condition of the blood pressure.

Antistreptococcic Serum.—Bouillon cultures of *Strept. pyogenes* are killed by heating, and injected into horses in increasingly large doses. Frequently, the killed cultures used in treating the horses are composed of several different strains of the streptococcus. In this case the resulting antistreptococcic serum is designated as "polyvalent," while the serum obtained after the injection of cultures consisting of but one strain of the organism, is called "monovalent" antistreptococcic serum.

In procuring the serum, handling, filtering, preserving and distributing for use, the methods are practically the same as those employed in the preparation of antidiphtheritic serum.

Antistreptococcic serum is carefully tested in regard to safety and freedom from microbial contamination. There are no methods available for definitely standardizing the product. The serum is often efficacious in cases of streptococcic infection.

Antigonococcic Serum.—Killed cultures of *M. gonorrhœæ* are injected intraperitoneally or intravenously into large, healthy rams, or other animals. The dosage is increased and finally live cultures are applied, the degree of immunity acquired being determined by complement fixation and agglutination tests of the serums from the animals

DORSET-NILES (ANTIHOG CHOLERA) SERUM (HYPERIMMUNE ERUM)*.—This product has been described in the preceding chapter nder "hog cholera vaccine" (Double Treatment). When the hyper-nmune serum is used unaccompanied by the virus, either among ealthy or diseased swine, the process is known as the "Serum-Alone lethod." Reichel† has succeeded in producing an antihog cholera erum which is sterile and free from inert solid matter by precipitat-ng the globulins. Dorset and Henley‡ have announced the produc-on of a clear and sterile serum by employing an extract of common arden beans together with salt to agglutinate the blood corpuscles.

ANTIRABIC SERUM.—Animals which have been immunized to rabies e bled and the immune serum may be used as a preventive and erapeutic agent. While this product is not often employed in prac-ce, yet it has been shown by various investigators that considerable otection is obtained from its use.

ANTIDYSENTERIC SERUM.—Experimental monovalent and poly-alent antiserums for epidemic dysentery have been developed by iga and Flexner, by the injection of horses with the filtrates from uillon cultures of the dysentery bacillus.

THE PRESERVATION OF ANTISERUMS.—The question of a proper eservative for antiserums has received much attention. The oblem of preservation involves several conditions, as the ideal pre-rvative, when incorporated in the proper volume of serum in efficient lutions, must possess marked inhibitive and germicidal power, it ust prove inert when injected into the patient, and it must produce objectionable precipitation of serum proteins. At present, trikresol purified cresols (0.4 of 1 per cent) is generally employed.

BIOLOGICAL PRODUCTS OTHER THAN VACCINES AND ANTISERUMS

TUBERCULINS.—*Koch's Tuberculin (Old)*.—Koch's tuberculin is the ncentrated, glycerinated, beef bouillon in which *Bact. tuberculosis* has en grown. The active substance of the tuberculin is apparently an buminous body insoluble in alcohol. The product is harmless for the n-infected, but exerts a toxic action upon tuberculous individuals, the action being characterized by a rise in temperature which begins two

* See U. S. Bureau of Animal Industry, Bull. No. 102.
† Reichel, Proc. 18th Ann. Mtg. U.S.L.S.S. Asso., 1915, p. 127.
‡ Dorset and Henley, Jour. Agr. Research, Vol. 6, May 29, 1916.

to ten hours after treatment, continues for a few hours and finally subsides. Tuberculin (old) is used as a diagnostic agent in both human and veterinary practice.

Tuberculin (old) is prepared from cultures of the human or bovine variety of *Bact. tuberculosis*. Apparently the active product can be obtained from attenuated as well as from virulent cultures. The organism is inoculated into beef bouillon to which 5 per cent glycerin has been added. The culture medium is usually distributed in flasks and the tubercle organisms, when inoculated, are carefully placed on the surface of the medium. The cultures are incubated at a temperature of 37° to 38° for six to ten weeks or longer, during which time a heavy growth slowly spreads over the surface of the medium and finally falls to the bottom of the flasks. In the successful preparation of tuberculin it is important that the cultures should remain undisturbed, having access to plenty of air, that the incubator temperature should be constantly maintained without fluctuations, and that the organisms should be allowed to grow until they have completely elaborated the active "tuberculinic" substance. After the growth is complete, the cultures are removed from the incubator and sterilized in streaming steam. The killed cultures are then evaporated over a water bath to one-tenth the original volume, the bacteria are removed by passing the cultures through paper and Berkefeld filter and a preservative is added. For cattle the dose of tuberculin concentrated by evaporation to one-tenth the original volume, is 0.25 c.c. to 0.7 c.c. Because of the fact that the material is thick and syrupy in consistency and the dose is inconveniently small, it is usually diluted with seven parts of weak carbolic acid solution. During the preparation it may be evaporated to four-fifths the original volume and preserved by the addition of 1 per cent carbolic acid of sufficient volume to dilute properly so that each dose is represented by 2 c.c. Two cubic centimeters of the diluted tuberculin is used as the dose for cattle. The product should be tested for activity by injecting known tuberculous animals with the tuberculin under test. The presence of typical reactions in tuberculous animals indicates the reliability of the product.

In human, as well as in veterinary practice, tuberculin may be applied as a diagnostic agent in various ways. In addition to the hypodermic injection of tuberculin (old), as described above, the method o

almette,* von Pirquet† and Moro‡ may be used in human practice. almette's ophthalmo test consists in the instillation in the eye of och's purified or refined tuberculin. Purified tuberculin is prepared y treating the original tuberculin with absolute alcohol, washing and ying the precipitate. One drop of a 1 per cent solution of purified berculin is placed in the eye. A positive reaction is manifested by a ngestion of the palpebral and ocular conjunctiva a few hours after the plication of the tuberculin. The method of von Pirquet* depends on the cutaneous application of the tuberculin. One drop of tuberlin (old) is placed on the arm, after cleansing the skin, and the small ea under the drop is scarified. Two or more small areas may be eated in this way, as well as a control area treated with sterile salt lution or a solution of glycerin and dilute carbolic acid in substituon for the tuberculin. The appearance of a reddish zone in from velve to twenty-four hours indicates a positive reaction. This area inflammation gradually increases somewhat in elevation and diameter d finally subsides in a few days. Moro's modification of von Pirquet's method consists in the use of tuberculin ointment prepared by the mbination of tuberculin (old) and anhydrous lanolin in equal parts. he ointment is vigorously rubbed on a small portion of the skin of the domen. A positive reaction is evidenced by the appearance of a stinct granular or papular eruption at the point of application after out twenty-four hours.

For the diagnosis of tuberculosis in cattle, the intradermal test is nerally regarded as next in importance to the older subcutaneous test. conducting this test 0.1 to 0.3 c.c. of a 50 per cent solution of tuberlin is injected into the cuticle layer of the skin at the base of the il. A positive reaction is present when, twenty-four to seventy-two urs after the injection of tuberculin, the localized area of skin shows circumscribed œdematous swelling.

Tuberculin (old) is usually distributed in small vials, sealed and beled. The labels should indicate the amount and dosage and the te of preparation. Under the influence of light and heat the fluid oduct may slowly deteriorate; therefore, when possible, it should be pt in the refrigerator until needed.

* Calmette, Presse Medicale, 1907, 15.
† von Pirquet, Berl. klin. Woch., 1907, 44.
‡ Moro, Münch. med. Wch., 1908.

Other Tuberculins.—Koch introduced tuberculin "T. R." (tube culin residuum) in 1897 and tuberculin "B. E." (bacillary emulsion) i 1901. The former is prepared by repeatedly centrifugalizing a suspen sion of the dried and ground tubercle organisms in water. The supe natant fluid "T. O." after the first centrifugalization is discarded an the final product consists of the constituents of the bacteria which ar insoluble in water. One cubic centimeter of the tuberculin "T. R. should contain the equivalent of 2 mg. of the dry tubercle solid Tuberculin B. E. is composed of a suspension of crushed or thoroughl ground tubercle organisms in 50 per cent glycerin solution. Eac cubic centimeter should contain the equivalent of 5 mg. of tuberc solids. Tuberculin T. R. and tuberculin B. E. are used as therapeuti agents, the latter probably being regarded with more favor by clin cians. The material is administered by subcutaneous injection, th time intervening between successive treatments varying from three t ten days. The initial dose recommended by most investigator is 0.0001 mg. or less.

MALLEIN.—Mallein is prepared from cultures of *Bact. mallei* b practically the same methods as those employed in manufacturin tuberculin from *Bact. tuberculosis*. The product is used for the diag nosis of glanders. A few hours after mallein is injected, subcutaneously into glandered horses a severe local reaction and a rise of temperatur usually follow. The thermal reaction is very similar to that produce in tuberculous animals by the injection of tuberculin. The local swel ing caused by mallein treatment is considered by some to be quite a diagnostic as the temperature reaction.

The ophthalmic mallein test, a comparatively recent method, whic was first used by Choromansky, appears to be attaining considerabl recognition as a valuable aid in diagnosis. The test consists in th application of concentrated mallein to the inner canthus of the eye A drop of the concentrated mallein in liquid form or a small bit of th same in desiccated condition may be used. In a positive case hyperemia and swelling of the conjunctiva and a purulent exudate a the inner canthus of the eye will appear from four to six hours after th instillation of the mallein.

Goodall* advocates the use of the intrapalpebral mallein test whic

* Goodall, Jour. Comp. Path. and Therap., 1915, Vol. 28, p. 281.

volves the injection of a small dose of mallein under the skin of the elid.

The stock culture of the glanders organism used in the preparation mallein should be one which possesses known virulent properties. is grown at a temperature of 37° for several weeks in flasks of glycerin uillon having a chemical reaction of about three points acid to enolphthalein. When the cultures are removed from the incubator ey are heated in streaming steam, passed through a Berkefeld filter d the filtrate is concentrated, preserved and distributed in labeled ls.

Suspensions for the Agglutination Tests

Agglutinins are hypothetical bodies existing in the blood and possi- other body tissues, of an individual affected with, or convales- t from, a specific infectious disease. The bodies possess the power o "clumping" and precipitating the specific bacteria which are the se of the disease in question. Thus, if a dilution of blood serum from a yphoid fever patient is mixed with living typhoid organisms, the s cific agglutinins present in the serum will cause the organisms to c se their motion and agglutinate or clump together in irregular sses. Normal human blood serum placed under the same conditions w fail to cause the agglutination of the organisms in similar dilutions. Te agglutination reaction may, therefore, be used in the diagnosis of c ain specific infectious diseases. The serum must be properly d ted in order that the reaction may be of diagnostic value, because u liluted, normal serum will cause a positive agglutination reaction in most cases.

The agglutination test is used as a practical aid chiefly in typhoid fe r in man and glanders in horses. The test may be conducted either m roscopically or macroscopically. In the microscopic method, the di ted serum from the suspected case is placed under the microscope w h the live, specific organisms in hanging drop. In the macroscopic m hod, the serum is added to an emulsion of the killed (heated) b teria in small test-tubes, and the resulting reaction detected with the n ed eye.

The emulsion, suspension or "test fluid" for the typhoid agglutina- ti test is prepared from a pure culture of *B. typhosus*. The organism is rown for twenty-four hours upon agar at a temperature of 37°.

The growth is then removed from the surface of the agar, placed sterile, physiologic salt solution and the organisms killed by heating a water bath at a temperature of 60° for one-half hour. The emulsi is then roughly standardized by adding sufficient sterile, physiolog salt solution to impart to the fluid the required degree of cloudines when compared with control emulsions. To the suspension of de typhoid organisms or "test fluid" a preservative, usually formali is added and the product is distributed in properly labeled bottles. conducting the test, the suspected typhoid serum is placed in sm tubes, each containing 1 c.c. of the suspension fluid, in such propo tions that the serum is diluted 1:50, 1:100, and 1:200. A floccule precipitate of the dead organisms indicates a positive reaction.

Suspension fluid for the glanders agglutination test is prepared practically the same manner as the typhoid test fluid. The glande organisms are grown on acid agar and the suspension fluid is usual preserved by the addition of carbolic acid. In conducting the glande agglutination test, the suspected serum is usually placed in the follo ing dilutions: 1:200, 1:500, 1:800, 1:1200, and 1:1,800.

The agglutination reaction has been applied experimentally a practically, with more or less success, in the diagnosis of Malta fev Asiatic cholera, bubonic plague, pneumonia, tuberculosis, contagio abortion (bovine) and other infectious diseases.

Substances Used for Diagnostic Tests

Luetin.—Noguchi* has developed a preparation known as *lue* which is used in the diagnosis of syphilis. The material is prepar from a number of strains of *Spirochæta pallida* grown, under anaerol conditions, on special ascites agar and bouillon media. After abunda growth of the spirochetes occurs, the agar cultures are ground and mix into a paste. To this material fluid cultures are added in sufficie proportion to form a liquid emulsion. The organisms are then kil by heating at 60° for one hour and a preservative is added.

In applying this diagnostic material to a suspected syphilitic c 0.05 c.c. is very carefully injected into, not beneath, the skin. An a on the antero-internal surface of the upper arm is usually chosen as t site of injection. A positive diagnosis of syphilis is indicated if, af

* Noguchi, Jour. Exp. Med., xiv, Vol. 16.

th third day a marked cutaneous eruption appears at the point of inculation.

ANTIGENS.—Certain antigens, such as gonococcus and syphilitic antigen, are of value for the purpose of conducting complement fixation tes in laboratory diagnosis. Gonococcus antigen consists of an extrt or filtrate prepared from a suspension of polyvalent gonococci. Syhilitic antigen consists of an extract prepared from either luetic ortain normal tissues such as beef or human heart muscle. Tubercusis antigen, as described by Craig,* consists of the filtrates of spially prepared cultures of *Bact. Tuberculosis*.

THE SCHICK TEST.—The susceptibility or non-susceptibility of individuals to diphtheria may be determined by the application of the te described by Schick.† For this purpose standardized diphtheria ton is required. 0.1–0.2 c.c. of a relatively fresh normal saline solutio containing $\frac{1}{50}$ minimum lethal dose of diphtheria toxin, for a 25g. guinea-pig, is injected intracutaneously. The appearance of a cumscribed area of redness at the site of injection after twenty-four toorty-eight hours indicates that the individual possesses practically nommunity against diphtheria.

Craig, Am. Jour. Med. Sci., 1915. 150, p. 781.
Schick, Munch, Med. Woch. 1913, 60, p. 2608.

DIVISION VI*

Microbial Diseases of Plants

Introduction

Although the earliest study of bacterial diseases in plants anteda the isolation of the tubercle bacterium and the cholera spirillum, t branch of bacteriology has not been marked by the progress which l characterized the investigation of animal diseases. The loss of a hun life or of a valuable domestic animal has appealed to the student disease more strongly than the blighting of a pear tree, or the wilting a potato vine, and, quite naturally, he has directed his efforts along th lines which have offered the greater inducements, and which ha demanded immediate attention.

However, with the introduction of new plants, foreign seeds, a strange nursery stock, many previously unheard-of plant diseases ha made their appearance. As the farming communities have beco more thickly populated, with less uncultivated land between the fiel these diseases have spread from farm to farm more rapidly than in earlier days, and the losses from these causes have been so heavy dur the past decade that the farmers, gardeners and orchardists have co to the Agricultural Experiment Stations all over the country for adv and assistance in combating their troubles. This has stimulated increased interest in plant diseases, especially along bacteriologi lines, with the result that to-day some forty bacterial diseases of pla have been described.

It is a matter of not infrequent observation that closely rela species of plants, as well as animals, exhibit a marked difference in th susceptibility to the same disease-producing agents. The Bartl pear, for example, suffers more severely from blight than the Kief and, among apples, the Toleman Sweet more than the Rome; the sm leaf, stemmy varieties of tobacco seem to be more resistant to the Gr ville wilt than the large-leaf kinds. Resistance of this sort, which

* Prepared by W. G. Sackett, except a protozoal disease "Fingers and Toes" by J. L. T revised by E. E. Tyzzer.

pers to be nothing other than a natural, inborn quality, may be designated as *natural immunity*, and it is immunity of this kind which plant breeding for disease resistance has secured. A good illustration of this is to be found in the wilt-resistant water melon of the Carolinas, which is the result of crossing a naturally susceptible water melon with a naturally resistant citron.

Acquired immunity in the plant world is a field yet to be explored. Cases have been cited in which *active immunity* appears to have followed the disease, but these are extremely rare and the evidence is very questionable. *Passive immunity*, at the present time, is unknown.

CHAPTER I

BLIGHTS

Stem Blight of Alfalfa

Pseudomonas medicaginis—Sackett

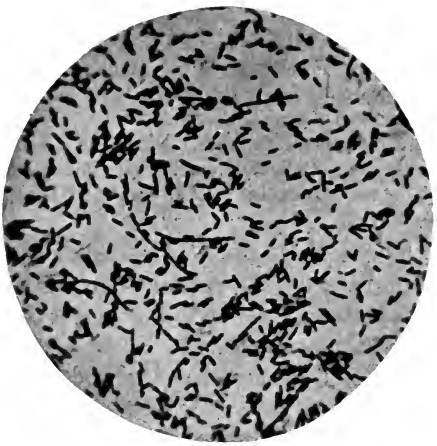
History and Distribution.—The disease has been known in Co rado since 1904 and was described briefly by Paddock in 1906 and m fully by Sackett in 1910. It is distributed generally over Colora , and is reported to occur in Utah, New Mexico, Nevada, Nebraska a Kansas.

Symptoms.—The disease is primarily a stem infection. In earliest stages, the stems have a watery, semi-transparent, yellowish olive green appearance along one side. Soon there oozes from the eased tissue a thick, clear, viscid liquid which spreads over the surf and collects here and there in little bead-like droplets. The exudate so dries in a short time with a glistening finish, and gives the stems v much the appearance of having been varnished, and where the liq has collected in little amber-colored scales and has hardened, it lo as if the varnish had run and dried. Stems in this condition have a d slightly rough feel to the touch. The exudate also dries uniformly o the surface or just beneath it, and there produces a dark brown, resin surface which blackens with age. Such stems are very brittle a easily broken, which fact makes it almost impossible to handle crop without an immense amount of shattering. The leaves attac to the blighted stems usually show the disease, and sometimes t exhibit the infection independent of the stem. In this case, petioles become watery and pale yellow, then droop. The mal may be confined to the petiole and base of the leaflet, or it may inv the whole of the blade. Occasionally leaves are found where inoculation has been made, apparently, in the margin of the leaf and the infection has proceeded toward the middle. In such instan the tender tissue has a watery look, as if it had been bruised.

One-year-old plants may exhibit blackened areas in the crown, and bck streaks which run down into the tap root. As the plant grows oer, this blackening increases until the whole crown becomes inved, and either the crown buds are destroyed or the root is no lger able to perform its functions, and the plant dies.

So far as our present observations go, the disease appears to run its crse with the first cutting, and those plants which have sufficient vlity throw out a good growth for the second and third cuttings.

CAUSE OF THE DISEASE.—If a small piece of the yellowish green, wery tissue from a diseased plant, or a fragment of the dried exudate islaced in a drop of clean water on a glass slide, there will appear on all



FI 144.—*Pseudomonas medicaginis.* Twenty-four hour culture on nutrient agar; stained with aqueous fuchsin; ×1000. (*Original.*)

sics of it, after half a minute, a dense, milky cloud, which can be seen reily with the naked eye, and which slowly diffuses out into the drop. Wen this preparation is examined under the low power of the microscce, this milky zone easily resolves itself into swarms of motile baeria.

The organism grows readily upon the ordinary culture media and pu cultures of the germ, inoculated into scarified stems of healthy alfal plants, produce the disease in seven to nine days with typical syptoms.

METHOD OF INFECTION.—Under field conditions the causal orga ism which, presumably, lives in the soil, enters the plants early in t growing season with soil through stems which are cracked and split late freezing. In some instances, inoculation appears to take place stomatal and water pore infections.

CAUSAL ORGANISM.—The writer has given the name *Ps. medicaginis* to causal organism, the characteristics of which are as follows: It is a short rod w rounded ends, about 1.2μ to 2.4μ by 0.5μ to 0.8μ the majority being 2.1μ by 0. It is actively motile by 1 to 4 bi-polar flagella; non-spore forming and non-caps forming. Filament formation occurs frequently. The organism stains reac with the aqueous stains, but is Gram-negative.

It produces a surface pellicle on broth. Shining, grayish white on nutrient ag becomes fluorescent green after three days. Gelatin stab, surface growth only, no liquefaction. Potato discolored, moderate growth, cream to light orange yell starch not destroyed. No growth in Cohn's solution. Good growth in Uschinsk solution. Plain milk shows no change. Litmus milk becomes bluer after se days, no curd and no peptonization in thirty days. No indol. No hydrogen s phide. Ammonia produced from asparagin solution, Dunham's solution a nutrient broth, but not from nitrate broth. Nitrates not reduced. No gas a no acid from dextrose, etc. Obligative aerobe. Optimum temperature 28°; growth at 37.5°. Thermal death-point 49.0° to 50.0°. Habitat, soil. Pathoge for alfalfa (*Medicago sativa*).

CONTROL.—The only practical way of combating and controlling t blight is by the introduction of resistant varieties, but no entir resistant strain has been obtained up to the present time, although t Grimm alfalfa is practically free from it.

As a means of control, the writer recommends that the frosted falfa be clipped, as soon as there is reasonable certainty that dan from late frosts is past. This will rid the plants of the diseased p tions, and afford an opportunity for the early growth of a new cutti If this is done in time, the regular number of cuttings should be secu with little or no loss in tonnage.

BACTERIOSIS OF BEANS

Pseudomonas phaseoli—Erw. Smith

Frequently the foliage, stems, and pods of the common beans, well as the Lima bean are attacked by a bacterial disease.

SYMPTOMS.—The pods and leaves seem to furnish the best fo supply for the microörganism, and it is here that we find the m

tpical lesions developing. Small, reddish spots appear which incase rapidly in size and develop into watery, amber-colored blisters, srounded by a pink or reddish border. These blisters are filled with nriads of bacteria, and in time, they dry down, forming a pale yellow oamber-colored crust over the affected areas. Ultimately the diseed leaves become brittle, ragged, and are worthless, while the pods cl, shrivel, and rot.

Method of Infection.—It is believed that the disease is introded with the seed, and when once established, is spread from plant tplant by rain, dew, and leaf-eating insects.

Causal Organism.—*Ps. phaseoli* Smith,* is a short, motile rod with rounded ers, which produces a characteristic yellow growth on the different culture media. Gatin slowly liquefied. Milk becomes slowly alkaline, casein is precipitated by la ferment and partially redissolved. Very marked diastatic action on potato stch. No gas from glucose, saccharose, etc. Aerobic. Uschinsky's solution, gwth feeble and retarded. Thermal death-point 49.5°.

Control.—Care should be taken to select seed from healthy fields were the disease has never occurred. The disease has been partially ctrolled by spraying with Bordeaux mixture when the plants were 2 3 inches high, again ten days later, and after blossoming.

Blight of Lettuce

Ps. viridilividum—Brown

The disease has been reported recently from the lettuce-growing sions of Louisiana, and is described as producing a shriveled, dried, bned aspect of the outer leaves, some of which may be in a soft, rted condition. The deeper leaves exhibit numerous separate or fsd spots with a water-soaked appearance; the center of the head is nt necessarily involved.

Causal Organism.—Miss Nellie A. Brown† has described the causal organism, *Psviridilividum,* as a short rod with rounded ends, motile by 1–3 polar flagella; sts readily with the ordinary stains; is Gram-negative. No spores have been obrved. In young agar cultures, the growth is cream-white mottled with yellow, thmottling disappearing with age. Gelatin is liquefied slowly. Nutrient broth

* Smith, Erw., Proc. Am. Asso. Adv. Sci., 46, 228–290, 1897.
† Brown, Nellie A., "A Bacterial Disease of Lettuce," Jour. Agr. Res., Vol. IV., No. 5, p. 5, 1915.

is clouded and becomes lime-green in color after ten days. On potato it produ a characteristic transient dark-blue green color which develops promptly and d appears on the sixth day or earlier. Growth develops readily in Uschinsky's a Fermi's solutions changing them to a pale green color in three to five days; fa growth occurs in Cohn's solution. Plain milk is cleared without coagulation, t cleared fluid becoming a pale turtle-green color; litmus milk becomes deeper bl Gas is not produced from the ordinary sugars in Dunham's solution. Nitra are not reduced, and some indol is formed.

Method of Infection.—Inoculation experiments indicate that infection may ta place either through the stomata or through wounds produced by mechani injury.

Control.—No control measures have been reported.

Blight of Mulberry

Pseudomonas mori—Boyer and Lambert (Smith)

History.—The disease was first studied in 1890 by Cuboni a Garbini in Italy, and later by Boyer and Lambert in France w named the causal organism *Bact. mori*, but did not describe it. 1908, Erwin F. Smith* found a similar disease in some of the Southe States, and described the causal organism.

Symptoms.—According to Erwin Smith, the blight attacks t leaves and young shoots of the mulberry, producing first water-soak spots, which later become sunken and black; "foliage more or l distorted; shoots soon show sunken black stripes and dead termi portions. Action of disease rather prompt." In very young shoo wood, pith and bark are invaded by bacteria; in older shoots the ger are confined mostly to the xylem.

Causal Organism.—The organism is a rod with rounded ends, 3.6μ by 1. motile by 1 to 2 polar flagella, attached to one end. No spores observed; pseu zoogloea occur. Stains readily with carbol fuchsin; Gram-negative.

On agar, spreading, smooth, dull, translucent, shiny, white; medium not stain

On potato, spreading glistening, smooth, white to dirty white, shiny, medi grayed, slight action on starch. Gelatin stab, filiform, no liquefaction. Beef bro pellicle, strong clouding. Milk, no coagulation, rendered alkaline, becomes clear solution of fat and casein, litmus not reduced. No growth or scant in Coh solution. Uschinsky's solution, copious, pellicle, not viscid fluid, bluish-fluoresc color. No gas from dextrose, saccharose, etc. Aerobic. No indol or slig Nitrates not reduced. Thermal death-point 51.5°; does not grow at 37°.

* Erwin F. Smith, Bacterial Blight of Mulberry, Science N S., Vol. XXXI, 803.

Blade Blight of Oats

Pseudomonas avenæ—Manns and *Bacillus avenæ*—Manns

History and Distribution.—A specific bacterial disease of oats has been described by Manns in 1909. What appears to have been a similar trouble, extending from the Atlantic coast west to Indiana, and from the Great Lakes to the Gulf States, was observed as early as 1890 by Galloway and Southworth. Its appearance was noted for the first time in Colorado in 1915.

Symptoms.—In the early stages of the disease there is "a yellowing, beginning either as small round lesions on the blade, or as long, streak lesions extending throughout the blade or even the whole length of the culm and blades. In the advanced stages, the affected blades take on a mottled to almost red color, which has been called 'rust' and 'blight.'"

Cause of the Disease.—The disease is produced by the symbiotic growth of two bacteria whose activity is favored by rainy, humid, and cloudy weather. One of these organisms, *Ps. avenæ*, alone, is said to be capable of effecting the blight in a mild form, while the other, *B. avenæ*, is nonpathogenic; but a mixture of the two germs results in an aggravated attack.

Method of Infection.—Infection takes place through the stomata, the organisms being spattered on the leaves from the soil by rains. Grain insects are also responsible for spreading the disease.

Control.—It is believed that the control of the disease lies in the selection of resistant strains.

Stem Blight of Field and Garden Peas

Pseudomonas pisi—Sackett

History and Distribution.—The disease occurs in several of the Western States, particularly in the mountain valleys of the higher altitudes. It was first observed in Colorado in 1915, where it caused a loss of approximately one-third of the field peas in the San Luis Valley, while in other parts of the State where garden peas are grown for canning purposes, the crop was materially affected.

Symptoms.—The plants usually show the infection before they are inches high, and many succumb before they reach that size. Both

* Manns, "The Blade Blight of Oats, A Bacterial Disease," Bull. 210, Ohio Exp. Sta., 1909.

field and garden peas are affected alike, and the symptoms simulate the bacterial stem blight of alfalfa. The stems have a watery, olive green appearance which soon becomes olive-brown, and in the last stages dark brown. The leaves and stipules appear watery at first, and if bruised, and later turn ocher yellow in color; this is often accompanied by wilting. In young plants, the discoloration of the stems is followed by a shrivelling, and ultimately the plants dry up and die; in the older ones, where the infection has taken place later, the same condition may result, but on the whole, the disease appears to be less serious, and in some cases the plants seem to outgrow the blight. Frequently when the first and earliest shoots are destroyed, the plant throws up new shoots from below ground, and a good late crop is obtained, in spite of the trouble.

CAUSAL ORGANISMS.—*Pseudomonas pisi*, n. sp., as described by Sackett, is a short rod with rounded ends, motile by means of a single polar flagellum; neither spores nor capsules observed; filaments formed commonly; stains readily with aqueous stains, and is Gram-negative.

It produces a flaky surface scum with heavy clouding in broth. On nutrient agar the growth is smooth, glistening, grayish white, and the medium is not discolored. Gelatin is liquefied rather rapidly. On potato, smooth, glistening, cream to orange-yellow; medium becomes grayish brown. No growth in Cohn's or Uschinsky's solutions. Heavy clouding with white surface pellicle in Fermi's solution; clouding with surface scum in Fraenkel's solution; slight, transient clouding in Naegli's solution. Plain milk is coagulated, and the coagulum is slowly peptonized, the supernatant liquid becoming yellowish green. Litmus milk becomes bluer, and the litmus is reduced, the liquid becoming greenish-gray. Neither indol nor hydrogen sulphide is produced. Ammonia is produced from asparagin and peptone. Nitrates are not reduced. No gas is formed from sugars, but acid is produced from dextrose, saccharose and galactose. Obligative aerobe. Optimum temperature 25° to 28°. Thermal death-point 50°. Habitat, soil.

Pathogenic for field pea and garden pea (*Pisum sativum* var. *arvense* and *Pisum sativum*).

METHOD OF INFECTION.—Experimental inoculations indicate that infections take place either through the stomata or through wounds produced by mechanical injuries.

CONTROL.—There seems to be a close relation between the prevalence of the disease and a late, cold spring. The low temperature appear to make the plants more susceptible, and as a result the early

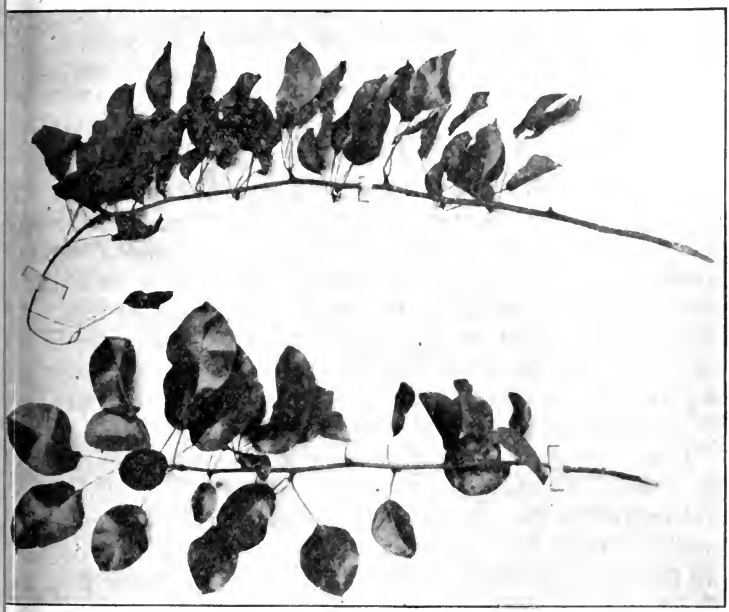
* Sackett, Walter G., "Stem Blight of Field and Garden Peas—A Bacterial Disease," Bull. 218, Colorado Exp. Sta., April. 1916.

antings are the worst affected. As a control measure, planting from o to three weeks later is suggested.

Pear Blight

Bacillus amylovorus—(Burrill) De Toni

History and Distribution.—As early as 1780, William Denning, fruit grower, who lived on the Highlands of the Hudson River, obrved pear blight in the trees of his neighborhood. It is very probable



G. 145.—Two pear twigs. The upper one affected with Fire Blight, the lower one healthy. (*After Sackett, Mich. Agr. Exp. Sta.*)

at blight existed many years before this in eastern North America on me of our native wild crabs, hawthorns, and wild plums, and with the troduction of cultivated varieties, it found a new field for attack. As e farming communities became more thickly populated, and the chards more numerous, it has spread gradually westward over the

Allegheny Mountains into the Mississippi Valley, across the Grea Plains, and over the Rocky Mountains to the Pacific Coast. So gen erally is it distributed over the United States and Canada that a blight free orchard is, indeed, a rare sight. The disease has progressed wit such severity that, to-day, commercial pear growing in Colorado ha been practically abandoned, and the industry in California is bein threatened with destruction. So far as our present knowledge goes the blight is of American origin and is confined to North America.

Occurrence.—While the ravages of the disease are worst upon th pear, from which fact the disease derives its name, many varieties of th apple, quince, apricot and plum, together with the mountain ash, servic berry, wild crabs and several species of hawthorn, have suffered severl from the same cause, and are capable of transmitting the disease fro one to the other.

Symptoms.—The disease is most easily recognized during the grow ing season, when it attacks the blossom clusters and the tips of th growing twigs. In this form it is known as *blossom* and *twig* blight. Th leaves attached to these parts usually turn brown or black, either whol or in part, the petioles blacken, and the young twigs show a blackene shriveled bark, having much the appearance of green brush which ha been burned only partially. It is from these symptoms that we g the name *Fire Blight*, so appropriately applied to pear blight. Th blackened, withered leaves cling tenaciously to their blighted twig long after the other leaves have fallen in the fall, and in this way affor the orchardist an easy way of recognizing the blighted areas.

Frequently the disease finds its way into the larger limbs and eve the trunk of the tree, where it produces *body* blight. This form characterized in the early stages by a cracking of the bark and th oozing of a thick, dirty white or brown, sticky liquid which collects he and there in drops over the injured surface. As the disease progresse the splitting of the bark increases and the area involved becomes roug giving rise to a canker. This is not to be confused with sun scald, i which the bark dries down and adheres firmly to the wood beneath, ar which is due to an entirely different cause.

The immature fruit manifests the blight by turning black, shrivelin and taking on a dried, mummified appearance. Accompanying the changes, drops of a thick, sticky exudate usually appear on the surfac

If a cross section is made of a diseased twig or limb, one invariab

fids a blackened ring in the region of the cambium layer. This penomenon, the significance of which will be explained later, serves aa reasonably reliable means of diagnosis.

Cause.—A microscopic examination of either the blackened cambm or a drop of the exudate shows swarms of motile rods, *B. amylovus*, which Burrill of the University of Illinois, as early as 1878, cdited with being the cause of pear blight. By inoculating healthy tes with this gummy material, he was able later to demonstrate his pnt experimentally, and with his work and that of a Dutch Botanist, Ukker, we have the begining of the study of bacterial diseases of pnts.

Methods of Infection.—The more careful observers believe that iects, especially bees, plant lice and twig borers are responsible for the itial infection and subsequent spread of the disease. It has been fnd that the bacteria find protection from the adverse conditions of vter in the margins of the old cankers next to the sound bark, and ao in some of the blighted shoots and twigs.* These hold-over bcteria become active with the increased flow of sap and the higher tnperature of spring, and soon spread into the adjacent healthy brk. Here they multiply so rapidly that at about the time† the trees a: in blossom, they begin to ooze from the cracks in the diseased bark a drops of a thick, sticky material, dirty white or brown in color. Isects are attracted to this ooze, apparently feed upon it, smear their ft, bodies and mouth parts, and then fly away to the opening bssoms. Here they feed upon the nectar and while so doing infect the wers. The germs increase rapidly in this sweet liquid, and each bee at visits the flower subsequently carries away millions of germs to fect other blossoms. From the flowers, the bacteria find their way to the cambium and softer tissues of the bark, where the disease is nfined almost entirely. After about ten days the progress of the rms can be noted by the blackening of the flower clusters, and the lting and blackening of the leaves of the fruit spurs. Following the llapse of the fruit spurs, the disease may move down the twig an ch or more a day, causing it to appear watery, turn black and shrivel. he blackening may be 10 to 12 inches behind the advancing infection.

* The writer examined a number of blighted pear twigs Apr. 14, 1911, collected from different chards in Colorado and found *B. amylovorus* alive in 23.53 per cent. The germs occurred in 2 cm. adjacent to the healthy part of the twigs.

† Whetzel, Bull. 272, Cornell Exp. Station, 1909.

This may continue until the whole limb becomes involved, but as a r
it is only the smaller twigs which are the worst affected. From t
it will be seen that the external blackening cannot be relied upon, ea
in the season at least, as a guide to the exact location of the disea
however, as the season advances, the plant tissues harden, conditic
for germ life become less favorable, and as a result, by the middle
summer, the active progress of the blight is checked by natural caus
and the blackening overtakes the advancing infection.

Blight which appears on the water sprouts of large limbs later c
usually be accounted for by inoculation by plant lice and the pear tv
borer.

CAUSAL ORGANISM.—According to Jones* *Bacillus amylovorus* possesses
following characteristics: Short motile bacillus, rounded ends, 1μ–1.8μ by 0.
0.9μ; stains readily with the aqueous stains; Gram-negative. No spores observ

Agar slant and potato, growth moderate, filiform, glistening, smooth, gray
white, semi-opaque, butyrous. Gelatin stab, growth rather slow, filiform, sli
crateriform liquefaction after twenty days. Nutrient broth, moderate cloudi
uniform; if left *undisturbed*, a delicate pellicle or ring may form which breaks up a
sinks with the slightest jar; scant finely granular sediment after ten days. Litn
milk, light blue in four days, pinkish in six days, light blue again in twelve da
upper layer blue in eighteen days; soft gelatinous curd six to ten days, with wl
on the surface. Cohn's solution, no growth. Uschinsky's solution, no grow
Nitrates not reduced. No indol. Thermal death-point 50°. Optimum te
perature 23° to 25°. Slight acid production but no gas from dextrose, etc. Sta
is not fermented.

CONTROL.—It is obvious that spraying is useless for a disease of tl
character, where the germs are located beneath the surface.

A systematic cutting out of the diseased limbs and twigs wherev
and whenever they appear is the only practical method of controlli
the blight. It is almost impossible to get all of the diseased mater
in the summer time when the heavy foliage hides it, but in the fall a
winter the blighted branches can be recognized very readily by the tu
of dead leaves clinging to them. It is necessary in removing the de
wood to cut well below the discolored part, 10 to 15 inches, f
the bacteria may be considerably in advance of the discolored are
Clean out all old cankers by cutting well into the healthy part and
removing the dried, diseased material. Disinfect the freshly cut su
faces of this wound as well as the exposed ends of twigs and limbs wi

* Jones, D. H., The Bacterial Blight of Apple, Pear and Quince Trees. Bull. 176, Onta
Agr. College.

1000 solution of mercuric chlorid. All diseased wood must be collected ad burned.

Streak Disease of Sweet Peas and Clovers

Bacillus lathyri—Manns and Taubenhaus

History.—The first recorded observations of this disease were made Diggs on sweet peas in Dublin, Ireland in 1904. The trouble was own locally as "Streak" disease of the sweet pea, and various rasitic fungi were assigned as the cause. One investigator even ntured the assertion that the malady was of a physiological nature. 1912, Taubenhaus isolated a bacillus from clovers and sweet peas lected in the vicinity of Newark, Delaware, and which bore lesions ilar to those described for "Streak." Subsequent inoculations h pure cultures proved the disease to be of bacterial origin and ntical with that observed in England and Ireland.

Symptoms.—The disease makes its appearance during the season of vy dew and is characterized by light reddish-brown to dark brown ts and streaks, almost purple when old, along the stems. They ually originate near the ground, which seems to indicate distri- tion by spattering rain and infection through the stomata. The ease is quickly distributed over the more mature stems, and ulti- tely the cambium and deeper structures are destroyed in con- uous areas resulting in the premature death of the plant. Occa- nally the petioles and leaves show the infection; the latter exhibit e water-soaked areas common to bacterial stomatal infections such are met with in alfalfa blight and bacteriosis of beans.

Causal Organism.—Manns and Taubenhaus have described the organism ch is responsible for the disease as a new species under the name *Bacillus lathyri*. s a small rod, motile by means of 8–12 short, peritrichiate flagella; it grows lux- ntly upon all of the common nutrient media, especially if sugars are present, ducing a yellow pigment; on glucose agar, colonies appear in twenty-four to ty-six hours, showing a tendency to become stellate or auriculate.

Pathogenesis.—*Bacillus lathyri*, n. sp. has been isolated from cific lesions on the following hosts: Sweet pea, Lathyrus spp., l, alsike and mammoth clovers, soy beans, garden beans, cow peas d alfalfa.

Method of Infection.—Infection appears to take place through e stomata, the organism being spattered on the plants from the l during rains.

CONTROL.—On small areas, heavy mulching of straw along eith side of the row is suggested as a possible means of preventing t distribution of the disease.

TOMATO BLIGHT

Bacterium (?) *michiganense*—Erw. Smith*

The disease is distinct from the wilt, caused by *B. solanacearum*, that there is not the sudden collapse of the whole plant, but rather slow yellowing or wilting of the leaves, one at a time. The caus organism produces cavities in the pith and bark as well as in t vascular system.

WALNUT BLIGHT OR BACTERIOSIS

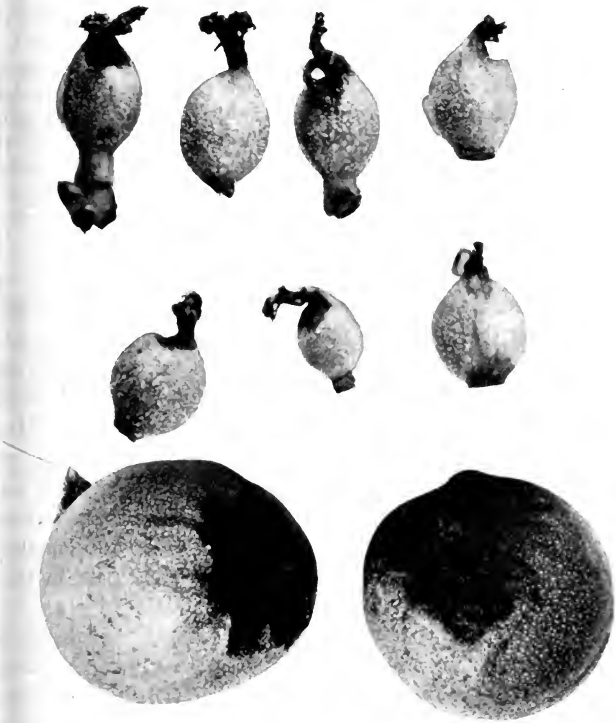
Pseudomonas juglandis—Pierce

HISTORY AND DISTRIBUTION.—Attention was first called to this d ease as it occurred in California by Pierce† in 1893 although it h been observed in Los Angeles County in about 1891. Outside California, it is known to occur in Oregon, Texas and midway do the Pacific coast of Mexico. What appears to be a similar troul has been reported from New Zealand and France.

SYMPTOMS.‡—All of the new, tender, growing parts of the tr such as young nuts and branches, petioles of leaves, midveins, fi lateral veins and adjoining parenchyma are subject to the attac On the branches, the disease always starts in the young succule growth and manifests its presence by small, discolored areas whi under favorable conditions may extend 2 to 3 inches along the gre shoot. As the infection progresses, the central portion of the lesi turns black and is surrounded by a water-soaked margin. In t later stages, the whole diseased area becomes blackened and in ma instances has a somewhat shrunken, dried-out, deformed, crack appearance due to the drying out of the tissue. In severe cases t tissue is killed inwardly to the pith, while in the milder attacks o the bark and wood are diseased. As the wood hardens, the infecti is checked, and the vitality of the tree is not affected to any extent,

* Smith, Erw., Science, N. S., Vol. XXXI, 803, p. 794, 1910.
† Pierce, N. B., Bot. Gaz., 31; 272–273, 1901.
‡ Smith, C. O., "Walnut Blight," Bull. 231, Calif. Exp. Sta., 320, 1912.

cp suffering rather than the tree. The leaves sometimes exhibit a bckening or browning of the petioles and veins, while the intermediate tiue may develop brownish, circular or angular spots. The disease ds not cause serious defoliation of the tree. The catkins are probably n affected. It is upon the young nuts that bacteriosis is especially



F. 146.—Walnuts affected by Bacteriosis, mostly stigma or blossom-end infection. (*After C. O. Smith, Calif. Bull.* 231.)

structive, and it would be of little economic importance did it not aack these. Many of the nuts may become infected and fall when ty are ⅛ to ½ inch in diameter and continue to drop throughout the smmer. A conservative estimate of the loss places it at 50 per cent i badly diseased groves. The most common point of infection is at t blossom end, although, it may start at any place on the nut. In

the early stage, the lesions appear as small, circular, raised, discolor
water-soaked areas; later, these spots increase in size and turn bla
Under favorable conditions, the disease may extend through the l
and shell-forming tissues into the kernel which at length beco
blackened and finally destroyed.

Causal Organism.—According to Smith, C. O., *Pseudomonas juglandis*, Pie
is a rod with rounded ends; single or in pairs, rarely in chains; measures 1.5μ to 3.0
0.3μ to 0.5 1μ; stains readily with the ordinary aniline dyes; Gram-positive; spores
capsules not observed; motile by means of a single polar flagellum; agar colo
nucleated, circular, moist, shining, pale yellow with regular margins; startif
liquefaction in gelatin; potato, abundant, moist, shining, slimy, raised, white ch
ing to yellow; uniform turbidity and ring in bouillon, slight flocculent precipit
indol produced; nitrates not reduced; enzymes, diastasic, cytohydrolytic, ren
proteolytic; milk coagulated, curd digested; litmus milk wine colored; viabi
nine and one-half months on potato; methylene-blue milk reduced.

Method of Infection.—It has been shown that the ca
organisms live over winter in the old lesions of the wood and bark
that in the spring they exude to the surface and are carried to the
growth, to which they gain entrance through the stomata. The dise
is most severe during seasons when the fogs and rainfall are heavi
and in those localities where rain and fogs are abundant. "Dur
one of these fogs the trees become saturated, water dripping from
portion of the tree to another which could easily carry the dise
organisms to healthy tissue." Distribution by this means is thou
to be one of the most important, if not the most important, method
spreading the trouble. Insects probably play some part in the
semination of blight.

Pathogenesis.—Pathogenic for Juglans regia (English) un
natural conditions; pure culture inoculations give positive lesions
Juglans nigra (eastern black), Juglans hindsii (nothern Cal. blac
Juglans californica (southern Cal. black), Juglans cinerea (butternu

Control.—Systematic spraying experiments with Bordeaux m
ture, lime-sulphur, and a sulphur spray have demonstrated that spr
ing is impracticable and has little value as a means of control. Appli
tions of lime to the soil have resulted in no benefit. It has b
observed that individual trees exhibit great differences in their natu
resistance to the blight, and at the present time the selection a
propagation of varieties which are more or less immune promi
the most practical solution to the problem.

CHAPTER II

GALLS AND TUMORS

Crown Gall

Pseudomonas tumefaciens.—Erw. Smith and Townsend

Crown gall is one of the most recent plant diseases to be traced to bacterial origin. Its occurrence is so common in nursery stock that in a certain Western State, 75 per cent of the young trees and shrubs condemned by nursery inspectors are condemned for crown-gall, and Tracey places the annual loss to orchardists at $500,000 to $1,000,000.

Fig. 147.—Crown gall with hairy root on nursery stock. Northern Spy apple. (*After Paddock.*)

History.—Smith and Townsend* working with the gall of the Paris daisy observed bacteria in these outgrowths in 1904, but it was not until 1906 that they succeeded in isolating the causal organism and

* Smith, Erw. F., and Townsend, C. O., "A Plant Tumor of Bacterial Origin," Science, N. S. Vol. XXV, No. 643, p. 671-673, 1907; "The Etiology of Plant Tumors," Science, N. S. Vol. XXX, No. 763, p. 233, 1909.

Townsend, C. O., "A Bacterial Gall of the Daisy and Its Relation to Gall Formations on Other Plants," Science, N. S. Vol. XXIX, p. 273 (Abstract), 1909.

in securing satisfactory re-inoculations. Subsequent studies* ha shown that this same microörganism is responsible for the pathologi condition that we recognize as crown gall in its various forms on different hosts. One of the remarkable things about this disease the large number of families which are subject to the infection.

PATHOGENESIS.—A partial list of the plants upon which crown occurs naturally or upon which it has been produced by laborat inoculation includes the daisy, tomato, tobacco, potato, carnati peach, rose, cabbage, grape, hop, sugar-beet, turnip, red beet, car radish, chrysanthemum, oleander, marigold, pyrethrum, almo clover, white poplar, Persian walnut, Pterocarya, gray poplar, cott alfalfa, raspberry, geranium, apple, willow, quince.

SYMPTOMS.—The swellings or galls, small at first, usually app just below the ground line (crown), at or near the juncture of the st and scion. These may be either hard or soft galls; the former smooth, soft, spongy, white to flesh-colored outgrowths which r reach a very appreciable size during one season and then be enti decomposed and disappear by the following spring; the latter incr in size more slowly, persist year after year, harden and become ro and warty on the surface with age. Both are crown galls and both produced by bacteria. According to Smith,† "Whether a crown shall develop as a hard gall or a soft gall would seem to depend chi if not altogether on which meristem cells receive the initial imp If the cells first infected are principally the mother cells of medul rays, we may assume that the gall will be a 'soft gall,' and rea inclined to decay. If, on the contrary, the needle or other carrie infection wounds principally those meristem cells which give ris tracheids and wood fibers, the gall will be a 'hard gall,' of slow gro and long duration." The structure of the galls is unlike that of c root of cabbage in that the latter is an hypertrophy while the for is an hyperplasia. Frequently this disease assumes a form know "hairy root" characterized by the presence of bunches or tufts of clo matted rootlets with enlargements at their bases. As the galls‡ enla the function of the adjacent conducting tissue is interfered with,

* Smith, Erw. F., Brown, Nellie A., Townsend, C. O., "Crown-gall of Plants: Its and Remedy," Bull. 213, Bur. Plant Ind., U. S. Dept. Agr., 1911.

† Smith, C. O., "Further Proof of the Cause and Infectiousness of Crown Gall," Bull Calif. Exp. Sta., 1912.

‡ Very hairy roots often accompany these.

e circulation is impaired, as is shown by the poor growth and dwarfed pearance of the trees.

The development of this disease is looked upon by Smith* and his sociates as paralleling closely what takes place in cancer in man and imals. The primary tumors have been observed to send out "roots" tumor-strands for some distance into the normal tissue, and from ese tumor-strands, secondary tumors may arise which tend to take the structure of the primary tumor, *e.g.*, "if the latter is in the stem d the former in a leaf, the secondary tumor shows a stem structure." here are no metastases in crown gall * * * for whether a cancer all be propagated by floating islands of tissue, or only by tumor-ands, appears to be a secondary matter depending upon the char-ter of the host tissues rather than the nature of the disease." The ient point is the internal stimulus to cell division which arises from e presence of the microörganisms within certain cells.

METHOD OF INFECTION.—Little is known about the natural channels infection, but inoculation through wounds induced by poor grafting reless cultivation, and by borers, nematodes, etc., is undoubtedly ponsible for many crown galls.

CAUSAL ORGANISM.—*Pseudomonas tumefaciens* is a short rod with rounded ends, tile by 1–3 polar flagella; measures 1.2 to 2.5μ by 0.5 to 0.8μ; neither spores r capsules demonstrated; pseudozoogloeæ occur; involution forms present; ins with the usual anilin stains; Gram-negative; on agar, slow, four to six days 25°; filiform, raised, white, glistening, somewhat slimy; potato, growth rapid, ite, smooth, wet-glistening; gelatin stab, filiform, no liquefaction; moderate, flat, form, white, smooth, glistening, no liquefaction; blood serum, moderate; broth, ring pellicle, clouding absent or inconspicuous; milk, coagulation delayed, curd not ptonized, litmus gradually blued then reduced; silicate jelly, slow white growth; hn's solution, scanty or absent; Uschinsky's solution, scanty, not viscid; NaCl uillon, 4 per cent inhibits, 3 per cent retards; bouillon over chloroform, growth restrained; no gas from sugars; ammonia is produced; nitrates not reduced; indol duction small; thermal death-point 51°; optimum reaction between +14 and 4 Fuller's scale; opt. temp. 25° to 28°, max. 37°, min. positive at 0°; killed dily by drying; moderately sensitive to sunlight; invertase and rennet thought be produced.

CONTROL.—Thorough inspection of nursery stock and care in the tivation of orchards not to wound the crowns are important factors.

* Smith, Erw. F., Brown Nellie A., McCulloch Lucia, "The Structure and Development of wn Gall; A Plant Cancer. Bull. 255, Bur. Plant Ind. U. S. Dept. Agric., 1912.

Plant on uninfected land and avoid heeling in healthy stock into so that has previously borne diseased plants.

Remvoing the galls results in no practical benefit.

Olive Knot.

Bacterium savastanoi—Smith*†

The olive knot has been known for many years, and is even de cribed by the early Roman writers; its bacterial nature, however, h been recognized only since 1886. It is most prevalent in those countri which border on the Mediterranean Sea, but it also occurs in the oli growing sections of California.

So far as is known, the causal organism enters the twigs and leav of the olive through wounds, and there produces roughened, wart-li swellings. The growth of the knots usually begins in the spring, a later in the season, if the trees are badly diseased, they show sca foliage, limited growth, and occasionally dead branches, especial where the galls have entirely encircled the twigs.

"Fingers and Toes" or "Stump Root" of Cabbages‡

Plasmodiophora brassicæ—Woronin

This organism which is classified as a rhizopod by many is t cause of a common disease of the roots of cabbages and of other cruc erous plants. The disease is sometimes called "fingers and toes" a it may cause much damage in market gardens. In it the roo are greatly hypertrophied appearing distorted and lumpy, like finge bent and swollen with rheumatism. The disease may be controll to some extent through the destruction, by burning, of all infect material as soon as the disease is recognized.

It is usually considered well to rely on the rotation of crops or, case the soil has become generally infested, to plant crops of anoth type for several years in order to prevent losses from this infectio The plants attacked are recognized by their stunted appearance a

* Smith, Erw., Bull. 131, Part IV, Bur. of Plant Industry, U. S. Dept. of Agriculture, 19

† Savastano, L. Les maladies de l'olivier et la tuberculose en particulier. Comp. Rend. 1144, 1116. Il bacillo della tuberculosi dell'olivo, nota suppletiva. Rend. Lincei 5:92 1889.

‡ Prepared by J. L. Todd and revised by E. E. Tyzzer.

e tendency of the leaves to wilt or turn yellow when an examination the roots will at once reveal the distinctive features of the disease.

The spores are liberated with the disintegration of the diseased roots d become disseminated in the soil during cultivation. Under appropriate conditions the spore is ruptured and a small flagellated, mœboid organism emerges. It is in this form that the parasites

G. 148.—Roots of Cabbage plant showing characteristic hypertrophy due to *Plasmodiophora brassicæ*. (*Woronin.*)

netrate the roots of the young plants in which they complete their velopment. The youngest forms seen within the vegetable cells ssess two nuclei each with a central mass of chromatin or karyosome. veral organisms frequently invade a single cell. As they grow there a multiplication of nuclei and the associated organisms tend to fuse gether to form plasmodia. Subsequently there occurs a series of anges, certain stages of which are readily distinguished but also

others which are more difficult to follow. The nuclei first lose tl greater part of their chromatin and appear pale and indistinct, whi attraction spheres appear at the opposite poles of each. The nucl then divide twice by karyokinesis and a small amount of cytoplasm separated off, constituting a gamete. The gametes now unite in pai and each pair becomes encysted to form a spore.

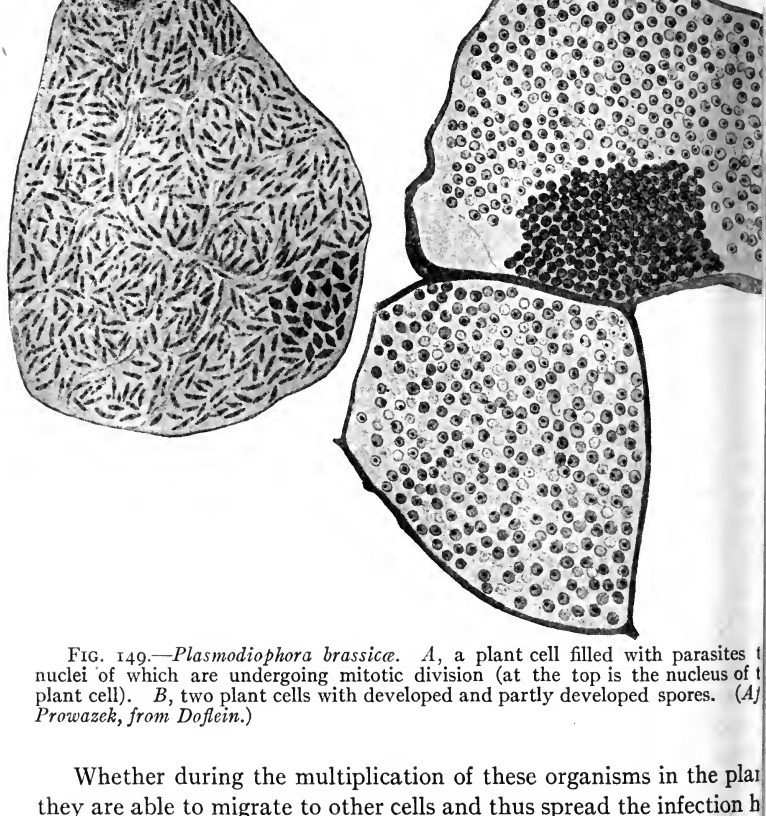


FIG. 149.—*Plasmodiophora brassicæ*. *A*, a plant cell filled with parasites t nuclei of which are undergoing mitotic division (at the top is the nucleus of t plant cell). *B*, two plant cells with developed and partly developed spores. (*A Prowazek, from Doflein.*)

Whether during the multiplication of these organisms in the pla they are able to migrate to other cells and thus spread the infection h been questioned. A number of investigators believe that the numb of infested cells is only increased by the division of the infected pla

ells which not only are greatly enlarged but also show evidence of roliferation in the presence of dividing cells. The hypertrophy of he plant cell is associated with hypertrophy of its nucleus and it is vident that the growth and increase of the parasite is favored by he reaction which its presence excites.

Tuberculosis of Sugar-beet

Pseudomonas beticola—Smith

History.—This new disease of the sugar-beet, resembling somewhat rown gall on the surface, but distinct from it, was first observed in he autumn of 1910 on beets from Colorado and Kansas.

Symptoms.—Affected beets bear numerous wart-like outgrowths or ubercles on the upper portion of the root. On section these show mall, water-soaked, brownish areas with more or less necrotic tissue n their interiors; such areas may develop small central cavities, and he softening may extend into the ungalled part of the beet; the dis-ased parts appear mucilaginous and stringy when touched, and under he microscope this broken-down tissue is found to be swarming with acteria.

Causal Organism.—According to Smith* *Pseudomonas beticola*, n. sp., is a otile rod with rounded ends, single or in pairs, chains or clumps; measures 0.6 0.8 by 1.5 to 2.0μ; flagella polar; no spores observed; capsule present; liquefies latin, but not blood serum; grows in beef bouillon containing 9 per cent NaCl; niform clouding and copious pellicle which falls easily in bouillon; thermal death-oint 51°; grows at 37° but best at 20°; grows slowly at 1°; produces a yellow m and pellicle in plain milk which is slowly coagulated; whey separates slowly; itmus milk is blued and later reduced; grows readily in Uschinsky's solution, scid; no growth in Cohn's solution; moderate growth on potato; does not roduce gas from dextrose, lactose, saccharose, maltose, mannite or glycerin; agar olonies, circular, smooth or wrinkled; indol is produced; grows in bouillon over loroform; resists drying; stains by Gram; is yellow or becomes yellow on all dinary media.

* Smith, Erwin F., "Crown Gall of Plants: Its Cause and Remedy." Bull. 213, Bur. Plant d., U. S. Dept., Agr., p. 194, 1911.

CHAPTER III

LEAF SPOTS

Citrus Canker

Pseudomonas citri—Hasse

The disease was probably introduced into the United States o nursery stock from Japan, and since 1912, has occurred in Florid Alabama, Mississippi, Louisiana, and Texas.

Symptoms.—According to Stevens,* citrus canker attacks a varieties of citrus trees of any commercial value in Florida, but it most severe on the grape fruit. Under field conditions a characteristi spotting of the fruit, foliage and twigs is produced which appears a small light-brown spots, 1.5 mm.–6 mm. ($\frac{1}{16}$ to $\frac{1}{4}$ inch) in diamete These spots may occur singly or several may coalesce to form an irregul area; they are raised above the adjoining tissue and are made up of spongy mass of dead cells, covered by a thin white or grayish membran which ultimately ruptures forming a ragged margin around the spo The fruit is especially susceptible to the infection, and drops soon after is attacked. The disease is spread rapidly from one part of the tree t another by insects, rains and heavy dews, so that when once infected, tree frequently becomes worthless in two or three months.

Causal Organism.—Miss Clara H. Hasse† has described the causal organis *Ps. citri*, as a short rod with rounded ends, motile by a single polar flagellum.

On nutrient agar, the growth is filiform, shining, dull yellow in color; on potat bright yellow, shining, viscid. In nutrient broth, a yellow ring is formed at t surface in old cultures. Litmus milk becomes deeper blue, and the casein is p cipitated. Gelatin is liquefied. Indol is not produced. No gas is formed fr sugars in Dunham's solution. Growth is slight in Uschinsky's solution, and nitrat are not reduced in starch nitrate solution. The organism grows best under aerob conditions.

* Stevens, H. E., "Citrus Canker, I, II, III," Bulls. 122, 124, 128, Fla. Exp. Sta., 1914, 191

† Hasse, Clara H., "*Pseudomonas citri*, the Cause of Citrus Canker," Jour. Agr. Res., V IV, No. 1, p. 97, 1915.

METHOD OF INFECTION.—Experimental evidence goes to show that infection takes place through stomata as well as through wounds produced by insects, or by other mechanical injuries.

PATHOGENESIS.—According to Berger, the following citrus varieties are subject to citrus canker: Pomelo, citrus trifoliata, wild lime, Navel, sweet seedlings, Satsuma, tangerine, King orange and lemon.

CONTROL.—Removal of the affected parts of the tree by pruning has proven a complete failure as a control measure, and the only practical means of handling the disease appears to be the prompt and complete destruction, by burning, of all stock that shows the slightest trace of infection.

ANGULAR LEAF-SPOT OF CUCUMBERS

Pseudomonas lachrymans—Erw. Smith and Bryan

HISTORY AND DISTRIBUTION.—The angular leaf-spot of cucumbers is a widespread disease occurring in many of the Eastern and Middle Western States. It has been recognized in the field for more than twenty years, but it was not until 1914 that the causal organism was isolated.

SYMPTOMS.—The disease is characterized by the "numerous, often confluent, angular, dry, brown spots which tear or drop out when dry, giving to the leaves a ragged appearance. In the early stages a bacterial exudate collects in drops on the lower surface during the night and dries whitish,"* and because of these tear-like drops of exudate the specific name *lachrymans* has been suggested for the causal organism. The young stems and petioles may become soft-rotted and crack open, but there is little evidence that the fruit itself suffers from the disease, other than indirectly from lack of nourishment resulting from the destruction of the active leaf surface.

CAUSAL ORGANISM.—*Pseudomonas lachrymans* is a short rod with rounded ends, motile by means of 1–5 polar flagella. No spores have been observed; capsules are formed on agar and in milk. It is Gram-negative and is not acid fast.

On agar, the growth is smooth, shining, transparent, white; agar colonies, two to four days old, exhibit opaque white centers which spread in radiating lines to the thin margin. Gelatin is liquefied slowly, and as the liquefaction progresses the upper part becomes stratiform, the lower part bluntly funnel-shaped. In

* Erw. F. Smith and Mary Katherine Bryan, "Angular Leaf-spot of Cucumbers," Jour. Agr. Res., Vol. V, No. 11, pp. 465, 475, 1915.

nutrient broth moderate clouding occurs, and a membranous pellicle is formed which breaks readily on shaking. On potato, the growth is slimy, shining, creamy white. Plain milk clears slowly without coagulation, becoming translucent and tawny-olive with age. Lavender-colored litmus milk is completely blued in three days, and a creamy-white pellicle is formed at the surface; clearing is complete in twenty days, and later the blue color bleaches out leaving the fluid a translucent brown.

The organism grows in Uschinsky's, Fermi's, and Cohn's solutions producing a green coloration in the first two.

No gas is formed from the ordinary sugars; acid is produced from saccharose and dextrose. Nitrates are not reduced. Hydrogen sulphid is not formed. A small amount of indol is produced in 2 per cent peptone water and peptonized Uschinsky's solution. Methylene blue in milk is rapidly reduced. The organism is an obligate aerobe.

Optimum temperature is 25° to 27°.; no growth at 36°.

Method of Infection.—The causal organism enters the leaves through the stomata, no wounds being necessary.

Control.—Laboratory experiments upon the germicidal action of copper sulphate on *Ps. lachrymans* suggest that Bordeaux mixture properly applied, may be a remedy for the disease.

Spot of the Larkspur

Bacillus delphini—Erw. Smith

So far as is known, this disease occurs only on the larkspurs of Massachusetts. Infection takes place through the stomata, resulting in numerous black spots on the leaves and stems.

Causal Organism.—Smith* describes the organism as a motile, gray-white non-liquefying, nitrate reducing bacillus. Agar colony has characteristic wrinkled structure. Grows in Uschinsky's solution. No growth at 37°; thermal death point 48° to 49.1°.

Bacterial Spot of Plum and Peach

Pseudomonas pruni—Erw. Smith

The first occurrence of the bacterial spot was reported on the Japanese plum in Michigan.† Later, what appeared to be the same disease was observed on the peach in Georgia‡ and Connecticut, and more recently it has been found throughout the South and Middle West.

* Smith, Erw. F., Science, N. S., Vol. XIX, No. 480, p. 418, 1904.
† Smith, Erw., Science, N. S., Vol. XVIII, 429, p. 456, 1903.
‡ Rorer, J. B., Science, N. S., Vol. XXIX, 753, p. 914, 1909.

SYMPTOMS.—On the plum, the leaves and green fruit exhibit numer-
ıs small, water-soaked spots; later the diseased tissue of the leaves
lls out, giving a shot-hole appearance, and the plums show black,
ınken areas and deep cracks. The spots may reach a diameter of
ıe-fourth to one-half inch.

On the peach leaves, angular, purplish-brown spots one-eighth to
ıe-fourth inch in diameter are formed, which drop out giving the shot-
ıle effect. The organism also attacks the young twigs and fruit. It
estroys the bark of the former, producing black, sunken areas, while
ı the latter it causes small purplish spots over which the skin cracks.

In both the plum and the peach, infection is believed to take place
rough the stomata. It is primarily a disease of the parenchyma,
ıt the vascular system is invaded ultimately.

CAUSAL ORGANISM.—*Ps. pruni* Smith, is a small rod, motile by one to several
lar flagella. It grows readily upon the ordinary culture media. On agar, it
sembles *Ps. campestris*, producing a distinctly yellow pigment, but is distinguished
its feeble growth on potato and by its growth in Uschinsky's solution, which is
nverted into a viscid material like egg albumin. Gelatin liquefied slowly. Casein
milk precipitated slowly and redissolved; litmus reduced but color restored later.
gas produced. Thermal death-point 51°.

DISEASE OF SUGAR-BEET AND NASTURTIUM LEAVES

Pseudomonas aptatum—Brown and Jamieson

HISTORY.—The bacterial leaf spot of sugar-beet and nasturtium
ıves was first observed in the summer and spring of 1908 on nastur-
ım leaves growing near Richmond, Va., and on sugar-beet leaves
tained from Garland, Utah; more recently the trouble has been noted
California and Oregon on the sugar-beet.

SYMPTOMS.—Affected nasturtium leaves exhibit water-soaked and
ownish spots from 2 to 5 mm. in diameter. The sugar-beet leaves
sclose "dark-brown, often black, irregular spots and streaks from
mm. to 15 mm. in diameter. They occur on the petiole, midrib, and
ger veins." Occasionally the discoloration extends along the veins,
d the tissue on either side is brown and dry; sometimes cork-like
otuberances occur at the central point of the spots. In badly dis-
sed petioles the tissue softens as though affected with a soft rot, but
ıere the infection is mild there is no indication of this condition.

Microscopic examination of the diseased spots and adjacent area sho the tissue to be filled with a large number of active bacteria. Sectio cut from the central portions of the diseased areas show the cell wa to be ruptured or collapsed, while the cells bordering the ruptur places show that the bacteria are in the cells. The disease is repr duced readily with typical symptoms by means of needle prick inocul tions with pure cultures. So far as has been observed, the caus organism does not attack the beet root, but is confined strictly to t beet leaf.

CAUSAL ORGANISM.—According to Brown and Jamieson,* *Pseudomonas aptatu* n. sp., is a short, motile rod with rounded ends; flagella, bi-polar; involution for rare; no spores or capsules observed; pseudozoögloeæ occur; aerobic; smooth whiti colonies on agar plate with fish scale-like markings; clouds beef bouillon in eighte to twenty-four hours; produces alkaline reaction in litmus milk, with a gradu separation of whey from curd; liquefies gelatin; produces ammonia; no reducti of nitrates; fluorescence greenish; no diastasic action on potato starch; grows Uschinsky's and Fermi's solutions; indol produced after ten days; optimu temperature 27° to 28°; maximum 34° to 35°; minimum 1°; thermal deat point 47.5° to 48°; vitality four to ten months in beef agar, ten to twelve mont in beef bouillon, depending on temperature; growth good on litmus-lactose ag growth much retarded on gentian violet agar; stains readily with basic anilin dy not acid fast; not stained by Gram; tolerates acids; oxalic 0.1 per cent; tarta 0.2 per cent; hydrochloric 0.1 per cent; tolerates sodium hydroxide in b bouillon, −18 Fuller's scale; no growth in Cohn's solution; killed readily by dryin not very sensitive to sunlight; retains its virulence two to three years.

PATHOGENESIS.—Pathogenic to nasturtium and sugar-beet leave spots have been produced by artificial inoculations on leaves of pepp lettuce, egg plant, and upon the leaves and pods of the bean plant.

METHOD OF INFECTION.—It is believed that infection takes pla only in bruised or wounded tissue, due to insects or to mechanical injur

CONTROL.—No practical methods of control have been undertake

* Brown, Nellie A., Jamieson, Clara O., "A Bacterium Causing a Disease of Sugar-beet a Nasturtium Leaves," Jour. Agr. Res., Vol. I, No. 3, p. 189, 1913.

CHAPTER IV

ROTS

Black Rot of Cabbage

Pseudomonas campestris—Pammel (Erw. Smith)

This disease is widely distributed in the United States and Europe d has become so serious on many truck farms that gardeners dread appearance as much as orchardists do pear blight. It is not confined cabbage, but it attacks other cruciferous plants such as cauliflower hlrabi, kale, rape, turnips, mangels, rutabagas and mustards.

Symptoms.—The first symptom is the withered, yellow margin of e leaf, giving the impression of a "burned edge." The progress of e disease is inward and downward through the vascular system, as indicated by the brown or black color of the veins and midrib. The sue of the vascular bundles is destroyed and the cell walls of the jacent tissue are dissolved, presumably by a cytolytic enzyme.* In s way practically all of the tissues are softened, disorganized, and a neral infection of the whole plant may follow. Diseased leaves fall matureły, leaving a long naked stalk with a tuft of leaves at the top. e dwarfed, one-sided growth of the heads, and in some cases the lure to produce heads is characteristic.

Method of Infection.—Water pore† infection along the margin the leaf is believed to be the most common method of entrance, hough root inoculation at the time of transplanting undoubtedly es place also. It has been shown, further, that the germ is intro-ed on the seed.‡

Causal Organisms.§—*Pseudomonas campestris* Pammel, is a short rod with nded ends, relatively shorter in the host tissue than on culture media, 0.7μ to

* Smith, Bull. 25, Bur. Plant Industry, U. S. Dept. Agr., 1903.

† Russell, Bull. 65, Wisconsin Exp. Station, 1898. Smith, Farmers' Bull. 63, U. S. Dept. Agriculture, 1898.

‡ Harding, Bull. 251, N. Y. Experiment Station, 1904.

§ For a means of distinguishing *Ps. campestris*, *Ps. phaseoli*, *Ps. hyacinthi* and *Ps. stewarti*, the student is referred to Bull. 28, p. 149, Div. Veg. Phys. and Path., U. S. Dept. Agr., 1901.

3.0μ by 0.4μ to 0.5μ; motile when young by one polar flagellum; no capsule de onstrated and no spores observed; zooglœæ in liquid cultures. Stains readily wi aqueous stains. Gram-negative.

It grows readily in the ordinary culture media. Upon potato, growth is chara teristic; at first light yellow, and in old cultures a golden brown, abundant, moi shining, slimy. Gelatin liquefied slowly. Litmus milk becomes slightly alkali casein separated and gradually redissolved. On nutrient agar, translucent, yell slime. No gas from dextrose, lactose, etc. Uschinsky's solution, growth retard and feeble. Aerobic. Indol produced. Nitrates not reduced. Diastase produc Optimum temperature, 25° to 30°; thermal death-point, 51.5°.

CONTROL.—The removal of diseased leaves in the early stages h been practised by some growers with success, but care must be tak not to remove so many that growth will be checked. Manure co taining diseased cabbage refuse must not be used. Seed disinfecti with 1:1,000 mercuric chloride, fifteen minutes, or formalin 1:2c twenty minutes, is recommended. Rotation of crops, and planting new land should be practised whenever possible. If practicable, t seed bed should be made in sterilized soil, so that the plants will healthy when set in the field.

WAKKER'S HYACINTH DISEASE

Pseudomonas hyacinthi—Wakker

HISTORY.—One of the earliest landmarks in the study of bacter diseases of plants is the excellent contribution of Dr. J. H. Wakker,* Dutch botanist, who between 1883 and 1888 published five papers or disease of the hyacinth, caused by *Ps. hyacinthi*. Erwin F. Smith† h carried the investigation farther and has described the causal organis more fully. The disease was first observed in the Netherlands where frequently causes serious losses in the hyacinth gardens. It is n known to occur in any other part of the world.

SYMPTOMS.—The disease is characterized by a yellow striping the green leaves and the bright yellow slime produced in the vascul bundles of the bulb. The infection in the leaf spreads slowly to t bulb by the multiplication of bacteria in the vascular system, filling t

* Wakker, Bot. Centralbl., 1883, 14, p. 315; Archives neerlandaises des sci. ex. et naturel Tome XXIII, pp. 18–20.

† Smith, Erwin F., "Wakker's Hyacinth Germ," Bull. No. 26, U. S. Dept. Agr., Div. V Phys. and Path., 1901.

vessels, especially those of the bulb, with a bright yellow bacterial slime. In time, the walls of the vessels are destroyed and large cavities are formed in the fibro-vascular bundles. The disease does not spread rapidly from bundle to bundle in the bulb, but is confined for a long time to the vessels first involved, a year or more being required for the destruction of the host plant. This is due, largely, to the resistance offered by the cells of the parenchyma to bacterial invasion.

METHOD OF INFECTION.—The causal organism enters through wounds in the leaves and through the blossoms, and when the disease is once established, it is probably spread by insects which visit the blossoms or eat the leaves. Daughter bulbs contract the infection from mother bulbs. Wakker believed the disease to be transmitted often by knives used around sick plants.

CAUSAL ORGANISM.—*Pseudomonas hyacinthi* Wakker, according to Erwin F. Smith, is a medium-sized rod with rounded ends, 1.0μ to 2.0μ by 0.5μ to 0.7μ, motile by one polar flagellum; non-spore forming.

It grows well upon the ordinary culture media, on most of which, as well as in the host plant, it produces a bright, chrome-yellow pigment. Gelatin and blood serum are liquefied slowly (six to seven days). Milk is rendered alkaline, and the casein is slowly precipitated. On nutrient agar, growth is copious, yellow, smooth, wet, shining, translucent, spreading. On 20 per cent cane agar, the zooglœa formed give the growth a papillose, verrucose appearance. Acid but no gas is formed in dextrose and saccharose broth; indol produced slowly. Nitrates not reduced. Feeble growth in Uschinsky's solution. Does not grow at 37°; optimum temperature 28° to 30°; thermal death-point 47.5°.

The hyacinth is the only known host plant.

CONTROL.—Diseased bulbs should be removed from the fields and destroyed; land on which the disease is present should be used for other crops; the use of infected tools without thorough disinfection should be avoided. The selection and breeding of disease resistant varieties, as advised by Wakker, suggests the most practical way of controlling the trouble.

BLACK LEG OR BASAL STEM ROT OF POTATO

Bacillus phytophthorus—Appel*

The disease is prevalent in the United States and Europe. It appears to originate in the seed tubers from which it extends upward

*Appel, Otto, "Untersuchungen u. d. Schwarzbeinigkeit. "Arb. Bio. K. G. Amt., Berlin, 1903

into the base of the stem causing it to turn black and rot. The vi grow spindling, turn yellow and die prematurely. The diseased tub may rot in the soil or later when in storage cause a soft rot of the cr

CAUSAL ORGANISM.—Erwin Smith describes the causal organism as a spore forming bacillus, motile by means of peritrichiate flagella. It stains with ordinary stains, but is Gram-negative. The growth is grayish-white on agar on gelatin plates large, round, white colonies develop promptly. Gelatin is liqu with funnel-shaped liquefaction. On cooked potato, white to yellowish gro Raw potato, white growth and black stain. There is a slow acid coagulatio milk with precipitation of casein and reduction of litmus. Thick pellicle heavy precipitate in potato juice. No growth in Cohn's solution. Mode production of hydrogen sulphide. Nitrates reduced. No indol. Acid f dextrose, saccharose, lactose, maltose and galactose. Some gas from ino lactose and mannite. Facultative anaerobe. Optimum temperature, 28° to Thermal death-point, 47°.

Closely related organisms are *B. solanisaprus* Harrison, and *B. a septicus* van Hall.

CONTROL.—In view of the fact that the germs are introduced v the seed potatoes, thorough disinfection of the seed with formali recommended.

BUD-ROT OF THE COCOANUT

Bacillus coli (*Escherich*) Migula

HISTORY AND DISTRIBUTION.—The bud-rot of the cocoanut been known for more than thirty years in Cuba and is to be found tributed more or less generally throughout tropical America and eastern tropics.

SYMPTOMS.—Johnston* states that in the acute stages of the dise the bud, or the growing point in the center of the crown, is affecte a vile-smelling soft rot which destroys all the younger tissues. M of the nuts fall, the lower leaves turn yellow and the middle folded undeveloped leaves die and hang down between the still green rounding ones. The rot gradually spreads from the base of one spik another until all are involved and shed their nuts; the leaf stalks bec so rotten at their bases that they are no longer able to maintain t natural position and droop or else fall off. From a central diseased t the infection may spread downward and into the trunk of the tree

* Johnston, John R., "The History and Cause of Cocoanut Bud-rot," Bull. 238, Bur. Ind., U. S. Dept. Agr., 1912.

a ort distance, rotting out the fundamental tissues and leaving only th fibers which are too hard to be disintegrated.

t has been estimated that in some cocoanut groves from 75 to 90 pe cent of the trees have been destroyed by the rot.

AUSAL ORGANISM.—*B. coli* (Escherich) Migula.

METHOD OF INFECTION.—It is believed that the causal organism en rs the host through insect bites or other mechanical injuries to the so tissue. Insects, birds or some form of animal life are held respon- sil for spreading the trouble.

CONTROL.—The removal of the diseased parts of a tree as well as sp ying have proved of no benefit in controlling the disease. "The ab lute destruction of diseased trees, a careful watch for the newly in cted cases, and their immediate removal has done much to prevent gr ter loss in the various regions."

BROWN ROT, A LEAF-DISEASE OF TROPICAL ORCHIDS

Bacillus cypripedii—S. Hori

HISTORY AND DISTRIBUTION.—The brown rot of orchids was first ob rved by Hori* in 1906 on orchids growing in the greenhouses in T yo, Japan. Since then the disease has been noted on orchids from F nosa grown in their natural habitat out of doors. In 1898 v. Peg- lio described a similar trouble in Italy which may be identical with th above.

SYMPTOMS.—The rot is characterized by dirty cinnamon or light ur er colored, depressed spots on the leaf-blade; these become darker wi age and may increase in size so rapidly that the entire green leaf is iscolored (yellowish) in a few days and dies. The rotting also sp ads downward into the stem, and if the diseased leaves are not re oved early, the entire stalk will be destroyed.

AUSAL ORGANISM.—Bacillus cypripedii is a medium-sized rod with rounded en ; single or in short chains; measures 1.5 to $2\mu \times 0.5$ to 0.7μ; stains readily with an e dyes; Gram-positive; motile by 4 peritrichiate flagella; non-spore forming; sm th, light grayish white colony, with pearl luster on agar; dirty cream colony

Hori, S., "A Bacterial Leaf-disease of Tropical Orchids," Cent. f. Bakt., Abt. II, Bd. 31, p. 1911.

v. Peglion, "Bacteriosi delle folie di Oncidium spec," Cent. f. Bakt., Abt. II, Bd. 5, p. 1899.

on potato; surface film on bouillon; liquefies gelatin rapidly; coagulates m ferments glucose with production of H and CO_2 in the ratio 1 : 3; indol positive a forty days; methylene blue reduced; ammonia and H_2S produced from bouill enzymes: amylase, oxidase, peroxidase; facultative anaerobe.

PATHOGENESIS.—Pathogenic to orchids grown in the hothouse a also in their habitat.

METHOD OF INFECTION.—The germs enter the leaf tissue chie through wounds caused by careless washing.

CONTROL.—Use only a soft sponge soaked in a 1 : 1000 solution mercuric chloride for wiping the leaves, and avoid excessive water as this favors the disease.

ROT OF CAULIFLOWER AND ALLIED PLANTS

Bacillus oleraceæ—Harrison

HISTORY AND SYMPTOMS.—This rot of cauliflower and allied pla was first reported in 1901 from truck gardens in the vicinity of Guel Ontario. It is characterized by a soft rot of the roots and a blacken of the stems and leaves. Harrison* has found this condition to traceable to an actively motile bacillus which invades the intercellu spaces of the plant and destroys the middle lamellæ.

CAUSAL ORGANISM.—*Bacillus oleraceæ*–Harrison is a rod with rounded er occurs single or in short chains; measures 2 × 0.6μ; motile by means of 7 to peritrichiate flagella; stains with the ordinary aniline dyes; Gram-negative; broth heavy turbidity and sediment, no pellicle; stratiform liquefaction of gela on agar spreading, thin, whitish, moist, slightly opalescent; neutral red agar change in color; litmus milk coagulated, soft curd slowly peptonized; blood ser slightly liquefied; growth positive in Uschinsky's and Fermi's solutions; potato wa straw-colored to moist, shining; opt. temp. 30°, max. 42°, min. 5°; thermal dea point 55°; facultative anaerobe; slight reduction of nitrates; indol slight; positive; slight gas from glucose and lactose, none from saccharose; acid fr sugars; enzymes: proteolytic, diastase, cytase (pectinase).

METHOD OF INFECTION.—Infection takes place chiefly throu wounds due either to mechanical or insect injuries. Warm weat combined with excessive moisture appears to favor the spread of disease.

* Harrison, F. C., "A Bacterial Disease of Cauliflower (Brassica oleracea) and Allied Plan Cent. f. Bakt., Abt. II, Bd. 13, pp. 46, 185, 1904.

PATHOGENESIS.—Pathogenic for cauliflower, cabbage, and turnips; a oft rot can be produced in a large variety of vegetables under labora- t y conditions by pure culture inoculations.

CONTROL.—Complete destruction of diseased crops by burning and c p rotation are to be recommended.

Harding and Morse,* from their extensive comparative studies of n roörganisms producing soft rots of vegetables make *Bacillus oleraceæ* o Harrison identical with *B. carotovorus* of Jones.

SOFT ROT OF CALLA LILY

Bacillus aroideæ—Townsend†

A soft rot of the calla lily, distinct from other soft rots, is scattered o r the calla-growing sections of the United States. The disease starts a he top of the corm and causes a rotting of the plant at or just below t surface of the ground. As a result the leaves and flower stalk turn b wn and fall over. The healthy corms are white, but the infected o s are brown, soft and watery.

It is believed that the causal organism lives in the soil and enters the p nts through wounds. The disease is undoubtedly spread from one l lity to another by shipping slightly diseased corms.

As a means of control, only sound corms should be used, and the s in the calla beds should be changed every three to four years.

SOFT ROT OF CARROT AND OTHER VEGETABLES

Bacillus carotovorus—Jones

A number of the cultivated plants of the north temperate zone, n ably those grown for their root crops, suffer, at times, from a bac- t al rot caused by a liquefying bacillus. Although probably as widely d ributed as any microörganism parasitic upon plants, it was not de- s bed until 1901.‡

Bacillus carotovorus is a wound parasite which invades the inter- c lar spaces, dissolving the middle lamellæ and portions of the inner

* See footnote, p. 624.

† Townsend, C. O., Bull. 60 Bur. Plant Ind., U. S. Dept. Agr., 1904.

‡ Jones, L. R., "A Soft Rot of Carrot and Other Vegetables," 13th Report Vermont Exp. St n, p. 299, 1901.

lamellæ, thereby establishing a condition which is known as a soft r
Jones* has shown this solution to be due to a bacterial enzyme whi
he has named *pectinase*.

CAUSAL ORGANISM.—The organism is a variable rod, majority 2.0μ by 0.
rounded ends, motile by 2 to 10 peritrichiate flagella; no endospores; no capsul
slight pseudozoogloeæ. Stains readily with aqueous stains. Gram-negative.

On agar, growth abundant, filiform to spreading, glistening, smooth, whi
opaque to opalescent. Potato—glistening, white, decided odor, smooth, butyro
medium grayed. Gelatin stab—filiform, liquefaction crateriform to infun
buliform, liquefaction begins second day and complete in six days. Broth—t
pellicle, clouding, abundant sediment. Milk—coagulated, slowly peptoniz
rendered acid, litmus reduced. Cohn's solution—no growth. Uschinsky's sc
tion—abundant growth. Quick tests; soft rot of uncooked carrots, turnips, c
bages. Slight gas produced from dextrose, lactose, saccharose, but not glycer
Acid from dextrose, lactose, saccharose and glycerin. Nitrates reduced. Sli
indol. Thermal death-point, 48° to 50°; grows at 37°. Optimum temperature
to 30°. Pathogenic to the roots of carrot, turnip, rutabaga, radish, salsify, parsn
bulb of onion, leaf stalk of celery, leaves and scapes of hyacinth, cabbage, ca
flower, lettuce, Irish potato, fruit of tomato, eggplant and pepper.

B. *oleraceæ* Harrison, and *B. omnivorus* van Hall, formerly described as bacte
species capable of producing soft rots, have been reported by Harding and Mor
as identical with *B. carotovorus* and therefore to be recognized no longer as disti
species.

CONTROL.—Jones believes that the soft rots can be practically h
in check by rotation of crops; by not using manure into which garc
refuse has been thrown; by drying the surface of the roots thorough
and exposing them to bright sunshine before storage; by maintainin
constant low temperature (4°) during storage.

SOFT ROT OF HYACINTH

Bacillus hyacinthi septicus—Heinz‡

A very active soft rot of the hyacinth bulb, producing a bad smelli
slimy condition in a few days, has been described by Heinz as caus
by an unpigmented, motile bacillus.

* Jones, L. R., "Pectinase, the cytolytic enzyme produced by *Bacillus carotovorus* a
certain other soft rot organisms." Tech. Bull. 11, New York Agr. Exp. Sta., 1909.

† Harding and Morse, Tech. Bull. 11, New York Exp. Sta., 1909.

‡ Heinz, Cent. f. Bakt., 5, p. 535, 1899.

Soft Rot of Muskmelon

Bacillus melonis—Giddings

History.—Toward the close of the season of 1907 the muskmelons i certain sections of Vermont were attacked by a soft rot. An i estigation of the cause of the trouble by Giddings* showed it to be c to a microörganism which he has called *B. melonis*.

Symptoms.—The decay usually begins on that part of the melon n t to the soil as shown by the shrunken but generally unbroken skin o r the soft diseased area. There is a complete collapse of the melons a ompanied by some frothing and a disagreeable odor in the last stages. A nicroscopic examination of the diseased tissue, both fresh and killed, s ws that the bacterial invasion is purely intercellular, and the patho- l ical condition of the tissue manifested as a soft rot is due to the s ution of the middle lamellæ.

Infection in the field appears to take place through wounds in the s n, and especially through cracks in the skin and flesh.

Causal Organism.—According to Giddings, *Bacillus melonis* possesses the f wing characteristics:

A bacillus 1.0μ to 1.7μ by 0.6μ to 0.9μ actively motile by 4 to 6 peritrichiate fl ella. Endospores not produced. Gram-negative. Stains readily with aqueous s ns.

In nutrient broth, strong clouding twenty-four hours, neither pellicle nor ring, sl t sediment. Agar stroke, abundant, contoured, shiny, glistening, without color, o escent growth having umbilicate elevation. Gelatin stab, infundibuliform li efaction in two days. Cooked potato, abundant, spreading, glistening, odor of d ying potatoes. Litmus milk, coagulated and reddened in three days, no di- g ion. No growth in Cohn's solution. Abundant growth in Uschinsky's solution, ri , pellicle and heavy sediment, odor of hydrogen sulphide. Vegetables rotted— m kmelon, citron, carrot, potato, beet† and turnip. Growth and some acid but n as from lactose, etc. Slight gas production from asparagin broth, abundant in f entation tubes of milk, this gas being 99 per cent carbon dioxide. Hydrogen s hide from nutrient broth and potato. Nitrates reduced. Slight indol. Am- m ia from asparagin broth; none from broth, gelatin, milk or urea. Thermal d h-point, 49° to 50°. Optimum temperature, 30°.

Control.—Spraying with Bordeaux mixture or other fungicides is r ommended as a preventive measure.

* Giddings, Bull. 148, Vermont Exp. Station, 1910.

† *B. carotovorus*, Jones, associated with several soft rots, does not rot the beet.

The melons should be supported by some means to keep them fr coming in direct contact with the soil, and should be supplied w adequate water during a dry season to keep them from cracking.

Soft Rot of the Sugar Beet

Bacterium teutlium—Metcalf

History.—A soft rot of the sugar beet, occurring in Nebraska, l been described by Metcalf and Hedgcock.*

Symptoms.—Beets affected with the rot show the lower half ba decayed and honeycombed with "pockets" or cavities filled witl slimy, stringy fluid, colorless, sour-smelling, and alive with bacte The vascular bundles remain intact, while the tissue surrounding th is usually consumed. Above ground the beets appear normal.

Method of Infection.—The germs gain entrance to the b through wounds and abrasions in the skin, and there is good reason believing that nematodes are responsible for many of the inoculatio

Causal Organism.—*Bacterium teutlium*, according to Metcalf, possesses e following characteristics:

It is a short, non-motile rod, rounded ends, 1.5μ by 0.8μ; neither capsules endospores have been observed; the organism stains readily with the aque stains. Gram-positive.

On nutrient agar, slow, scant, translucent, porcelain white, non-viscid, and pe trates the agar. On cane-sugar agar growth more rapid, viscid, watery, vitreou translucent, colorless. Gelatin stab—scant, filiform to beaded, dirty white, liquefaction. Cane sugar gelatin—characteristic cumulus cloud appearance stab, no liquefaction. Nutrient broth—slight clouding and sediment, acid p duced. No evidence of growth in milk. No visible growth on potato. On car clear, viscid and acid. On sugar beet, viscid, clear, spreading, copious, a parenchyma destroyed leaving vascular tissue. No growth in Uschinsky's, Fern Pasteur's, Fraenkel's or Dunham's solution. No gas from dextrose, sacchar etc. Facultative anaerobe. No growth at 37°. Optimum temperature, Thermal death-point, 45°.

Control.—The rot is less apt to be serious if the beets are grown relatively dry soil and if rotation of crops is practiced. The select of resistant varieties seems to be the most practical solution of problem.

* Metcalf and Hedgcock, "A Soft Rot of the Sugar Beet," 17th Annual Report, Nebr Agr. Exp. Sta., pp. 69–112, 1904.

CHAPTER V

WILTS

Wilt of Cucurbits

Bacillus tracheiphilus—Erw. Smith

History and Distribution.—The bacterial wilt of the muskmelon, ıcumber, squash and pumpkin was first reported by Erwin Smith* in 3ọ3. It is widely distributed over the United States east of the Rocky Iountains and seems to have different host preferences in different ıcalities.

Symptoms.—The disease is characterized by a wilting of the vine, ıre and simple, without any visible external cause such as mildew, ıst or leaf spot. The leaves and runners wilt suddenly as if from lack water or too hot sun, the runner becoming prostrate on the ground. rom two to three days usually elapse before the wilting of the whole ne is complete, and it may remain in this wilted condition for several ıys, after which the leaves begin to dry up, but retain their green ılor for considerable time. One runner may die at a time, beginning the tip and working back toward the root, after which a general fection is to be expected. If inoculation takes place upon the main em, several or all of the runners may show the wilt at the same ne.

The disease is caused by a bacillus whose growth fills the water ducts tracheæ with a white, viscid material which prevents the rise of ıter, and wilting follows. If the severed ends of a diseased vine are bbed together gently and separated slowly, this sticky liquid will ring out in fine threads 2 to 3 cm. in length.

Method of Infection.—Under field conditions, the disease is read principally by insects, especially the striped cucumber beetle d the common squash bug.

* Erwin Smith, Cent. f. Bakt., Bd. I, II., Abt., pp. 364–373. 1895.

CAUSAL ORGANISM.—Erwin F. Smith describes *Bacillus tracheiphilus* as a ro 1.2μ to 2.5μ by 0.5μ to 0.7μ, actively motile when young.

Growth occurs on the ordinary media. Upon agar, the growth is milk-white an extremely viscid. Upon potato, a gray film is produced, much like that of *E typhosus;* the potato is unchanged. Gelatin is liquefied and no change occurs i milk. Acid but no gas is produced in saccharose and dextrose broths. The orgar ism is aerobic and possibly facultatively anaerobic. Optimum temperature between 20° and 30°. No growth at 37°. Thermal death-point, 43°.

CONTROL.—The same precautions and preventative measures are t be recommended for the wilt of cucurbits as are given for tomat blight.

WILT OF SWEET CORN

Pseudomonas stewarti—Smith

The early varieties of sweet corn grown in the truck gardens of Lon Island* are subject to a bacterial disease which manifests itself by wilting and drying up of the leaves. It also occurs in Iowa, and has been reported from certain parts of New Jersey.

The wilting may occur at any stage of growth, but the plants seer to be more susceptible at the time of flowering. As a rule the leave succumb one at a time, although on the younger plants they may a wilt simultaneously. There is no external evidence which woul indicate the cause of the trouble, but if a diseased stalk is cut lengtl wise, the fibro-vascular bundles appear as yellow strands in the whit pith. A cross-section of such a stalk will show drops of a yellow visci substance, composed largely of bacteria, exuding from the cut ends of th bundles. The infection is not confined to the stalks but can be foun in the vascular system of the leaves, husks and cobs as well. Th vessels are the principal structures invaded, but in time small cavitie filled with the bright yellow slime are formed in the surroundin parenchyma.

METHOD OF INFECTION.—The germ may enter its host throug either the roots, stomata or water pores and when once inside the va cular system, it multiplies very rapidly and fills the water tubes with yellow slime and wilting follows.

CAUSAL ORGANISM.—The organism was first described by Stewart and lat named *Pseudomonas stewarti* by Erwin Smith.

* Stewart, F. C., "A Bacterial Disease of Sweet Corn," Bull. 130, N. Y. Agr. Exp. Sta., 189

It is a short, relatively thick, motile rod with rounded ends; occurs usually in airs. No endospores observed. Stains readily with the aqueous stains.

It grows well upon the ordinary culture media. On agar, smooth, shining, ellowish-white to deep yellow, lobate. On potato, spreading, deep yellow be-oming slightly iridescent, smooth; potato is browned. Broth—thin film, slight louding and slight flocculent white precipitate. Milk—slight peptonization with-ut coagulation; litmus reduced. No gas is produced from dextrose, etc. Good rowth in Uschinsky's solution. Facultative anaerobe. Pathogenic for sweet orn.

CONTROL.—It is believed that the germ is disseminated on dis-ased seed and therefore disinfection of the seed before planting is ecommended.

The disease is also spread by the use of manure which contains iseased stalks.

Varieties differ considerably in their susceptibility, and by the election of the more resistant kinds some relief can be secured.

Rotation of crops and planting on new land, when available, should e practised.

Field corn and pop-corn are not affected by the wilt.

WILT OF TOMATO, EGGPLANT, IRISH POTATO AND TOBACCO

Pseudomonas solanacearum—Erwin Smith

HISTORY.—A bacterial wilt affecting a number of plants of the otato family has been described by Erwin Smith.* The disease was rst observed in the Atlantic coast and southern states. In 1903 tevens† and Sackett described a wilt of tobacco in Granville County, .C. and this, too, Smith‡ has shown to be due to the tomato wilt rganism, *Ps. solanacearum*. Quite recently Miss Bryan§ has shown ne same organism to be the cause of nasturtium wilt.

SYMPTOMS.—The disease usually manifests itself by a sudden wilting f the foliage, and, as a rule, with little or no yellowing. This may be dicated at first by the collapse of a single leaf, but in time the whole lant will succumb. Following the wilting, the parts affected shrivel,

* Smith, Erwin F., "A Bacterial Disease of the Tomato, Eggplant and Irish Potato," Bull. , U. S. Dept. Agr., Div. Veg. Phys. and Path., 1896.

† Stevens and Sackett, "Granville Tobacco Wilt," Bull. 188 N. Car. Exp. Sta., 1903.

‡ "Granville Tobacco Wilt," Bull. 141, U. S. Dept. Agr., Bur. Plant Industry, 1908.

§ Bryan, Mary K., "A Nasturtium Wilt Caused by *Bact. Solanacearum*," Journ. of Agr. esearch, Vol. IV, No. 5, p. 451, 1915.

turn yellow, then brown, and finally black. If a diseased stem is split lengthwise, black streaks, following the fibro-vascular bundles, can be traced the whole length of the stem and often out into the corresponding leaves. The vessels are packed with bacteria which ooze out on the cut surface as little drops of a dirty white, slightly viscid liquid. The bacillus destroys the parenchyma of the pith and bark and mechanically plugs the water tubes so that the water supply from the soil is shut off and wilting follows. In the tubers of the potato, the rot begins in the blackened vascular ring and spreads in all directions, producing well-defined cavities next to the ring.

METHOD OF INFECTION.—Insect enemies are largely responsible for the spread of the wilt, especially above ground, while beneath the surface inoculated soil enters the roots through wounds made either by transplanting, cultivating, or nematodes. In the case of the nasturtium, stomatal infections have been demonstrated.

CAUSAL ORGANISM.—According to Smith, *Pseudomonas solanacearum* is a medium-sized rod, rounded ends; 1.5μ by 0.5μ; motile by a single polar flagellum zoogloeæ formed in liquid media; stains readily with aqueous stains.

Zoogloeæ produced at the surface in beef broth, copious dirty white sediment reaction made alkaline. Casein of milk dissolved without precipitation and medium becomes alkaline. On nutrient agar, growth is smooth, wet shining, slightly viscid at first dirty white becoming yellowish, then brown; agar browned. Gelatin stab—growth best at surface, pure white, smooth, wet shining, no liquefaction or very feeble after six weeks. Potato—wet shining, not wrinkled, copious, dirty white and later brown to black; medium browned. Neither acid nor gas produced in any of the culture media or from glucose, etc. Obligate aerobe; ammonia produced in nutrient broth and potato tubes; pigment formation aided by glucose fructose and saccharose. Grows well at 37°. Thermal death-point, 52°.

PATHOGENESIS.—Pathogenic for tomato, potato, eggplant, tobacco Jamestown weed, black nightshade, physilis, petunia and nasturtium.

CONTROL.—If the disease is not too general, it is possible to control its spread by removing the dead plants and burning them; the early and complete destruction of all insect pests is important; if available and practical, new land or land which has not been planted to any of the potato family for a period of years, should be used; only those seeds and tubers which have come from plants grown in localities free from the disease should be planted; the use of infected manure or soil should be avoided.

Additional Bacterial Diseases

Angular Leaf Spot of Cotton, *Pseudomonas malvacearum* Smith.*
Gum Disease of Sugar Cane, *Pseudomonas vascularum* Cobb,† Smith.‡
Leaf Spot of Broom Corn, Burrill.§
Bacteriosis of Tomatoes, *Bacillus briosii* Pavarino.‖
Wilt of Banana and Plantins, *Bacillus musæ* Rorer.**
Bacteriosis of Ixia maculata, *Bacillus ixiæ* Severini.††
Bacteriosis of Gladiolus colvilli, *Pseudomonas gladioli* Severini.††
Bacteriosis of Orchard grass, *Bacterium rathayi* Smith.‡‡
Rot of Potatoes, *Bacillus solanisaprus* Harrison.§§
A Bacterial Disease of the Mango, *B. mangiferæ*‖‖ Doidge.

Smith, Erw., Bacteria in Relation to Plant Diseases, I, p. 95, 126.
Cobb, N. A., Rept. New So. Wales Dept. Agr., 1893, pp. 1–21.
Smith, Erw., Cent. f. Bakt., II Abt., Bd. XIII, 22–23, pp. 726–729, 1904.
Burrill, Bull. 6, Ill. Exp. Sta., pp. 165–176, 1889. Smith and Hedges, Science, N. S., Vol. XXI, 535, p. 502, 1905.
Pavarino, G. L., Atti R. Accad. Lincei. Rend. Cl. Sci. Fis., Mat. e Nat., 5, ser., 20 (1911), No. 5, pp. 355–358.
* Rorer, J. B., Phytopathology, I, (1911), No. 2, pp. 45–49.
† Severini, G., Ann. Bot. (Rome), 11 (1913), No. 3, pp. 413–424.
‡ Smith, Erw. F., "A New Type of Bacterial Disease." Science, N. S., Vol. XXXVIII, No. 991, p. 926, 1913; Sitz. Ber. Weiner Akad., 1 Abt., Bd. CVIII, p. 597.
§ Harrison, F. C., "A Bacterial Rot of the Potato Caused by Bacillus solanisaprus." Cent. f. Bakt., Abt. II, Bd. 17, p. 34, 1907.
‖ Doidge, Ethel M., Annals of Applied Biology, Vol. II, No. 1, May, 1915, pp. 1n45.

DIVISION VII

Microbial Diseases of Insects

Introduction*

Microbial diseases are of interest to the layman from two econon standpoints:

I. At certain stages of their existence, certain insects ha an economic value; for this reason their breeding is desirable and a plague which devastates their numbers should be combated. P teur was the pioneer in this line, not only being the discoverer of t first known bacillary insect disease, flacherie of the silk-worm, but worked out an efficient method for its scientific control. His work the more notable since he was handicapped by the lack of suital methods of isolation and study of the organisms discovered.

II. Certain insects or their larvæ are at times veritable plagu laying waste valuable crops and causing serious hardships, ev famine and epidemic disease resulting in many cases. Not infrequent these insects naturally become subject to microbial enemies which ma heavy inroads on their numbers, thus checking the insect plague. Su an epizootic occurred among the white grubs in Michigan in 1912.

The artificial employment of these microbial enemies natura suggests itself as a means of voluntary control, and such experimer have been carried out successfully on a practical scale. One of t best examples of this is seen in the arrest of the locust epizootic in Mexi and the Argentine Republic by the use of cultures of *B. acridiorum.*

Another thing worthy of note which has been mentioned many tim by those working with microbial insect diseases, is the fact that the diseases seem to be almost explosive in character; an epizootic amo

* Prepared by Zae Northrup, except paragraphs on "Miscellaneous Fungus Diseases" C. Thom.

sects caused by a fungus disease is after a comparatively short time ntirely wiped out and another disease takes its place; in many places acterial diseases seem to have almost entirely supplanted the fungus seases. This succession of diseases among insects takes place with ch periodicity that those who are most intimately connected with eir study, can predict very closely both the duration of the epizootic progress and the time intervening before the onset of the next one. his same periodicity takes place more or less among the more highly ganized animals but the "explosive" character is greatly modified the length of the life cycle.

Miscellaneous Insect Diseases

Bacillus erausquinii n. sp. was isolated from locusts of the species *omalea miles* in Argentina by Cullen and Maggio. It is said to have any characteristics which distinguish it from *B. acridiorum.*

A disease of the caterpillars of *Gortyna ochracea,* and artichoke pest, recorded in the Department of Var, France. *Bacillus gortynæ* was olated as the causal factor.

Bacillus pyrameis I and II were isolated from the blood and tissues the caterpillars of *Pyrameis cardui,* another artichoke pest. These ay be distinct or merely varieties of a single species; they may repre- nt one or more saprophytic species widespread in nature which are adily adaptable to a parasitic life (Paillot).

Two associated microörganisms, one a motile rod and the other a ccus were the cause of epizootics destroying nearly all of the cater- llars of *Galleria melonella,* the bee moth, which were being raised for perimental purposes (Metalnikov). The rod form was the more viru- nt on injection. The manner in which infection takes place was not termined.

Saprolegniaceæ and Entomophthoraceæ.*—Some of the *Sapro- gniæ* (all water fungi) form conspicuous masses of mycelium around ad insects in stagnant water. The *Entomophthoraceæ* are parasites insects on land. One of these, *Empusa muscæ,* destroys the common use-fly, which, after death from this disease, is found attached by its outh parts to windows or woodwork.

* Prepared by Charles Thom.

ENTOMOGENOUS FUNGI.*—The practical usefulness of some of thes species, notably *Sporotrichum globuliferum*, as a chinch-bug disease, ha been studied carefully. While the work was markedly successful i causing an epidemic disease when conditions favored it, dependenc upon particular conditions was so complete that the production of th disease as an effective destroyer of pests failed. Similar results hav attended the effort to use other fungi as insect-destroyers. The condi tions which make possible their development in epidemic form onl occur occasionally. These conditions in themselves are, as a rul very unfavorable to insects. Under other climatic conditions, thes diseases appear only as isolated cases, negligible in their effect upo the insect population, no matter how carefully the inoculatin material is spread by man.

BACTERIAL DISEASE OF JUNE BEETLE LARVÆ, *Lachnosterna* sp *Micrococcus nigrofaciens*—Northrup†

HISTORY AND DISTRIBUTION.—The characteristics of this diseas were noted in 1893 by Krassilstschik, Russia, but he did not conside it a disease. It is common everywhere in the United States tha white grubs of this and related species are found; infected specimer have also been received from Porto Rico.

SYMPTOMS.—The normal larva is white, quite firm, covered wit conspicuous hairs; the head is brown as are also the spiracles or breath ing pores along either side. The diseased larva has black shiny spot sharply circumscribed, located mainly along the joints of the leg spiracles, and upon the dorsal or ventral segments of the white portio

Badly diseased larvæ are almost entirely black or brownish blac in color; the whole body often seems to be in a state of advanced putr faction, yet the larva still shows life.

The progressive destructiveness of the disease is most marked i the affections of the legs. In some cases the infection begins at the ti of the leg and as it progresses, the leg, segment by segment, blacker and drops off, leaving the stumps shiny, black, and sometimes swolle in appearance; in other cases the infection occurs at one of the inte mediate joints or at the joint nearest the body of the grub, the leg i

* Prepared by Charles Thom.

† Northrup, Z. A bacterial disease of June beetle larvæ, *Lachnosterna* spp. Tech. B 18. Mich. Exp. Sta., 1914.

tir loosening and breaking off at the point of infection. Within ce ain limits neither the size nor the number of infected areas seems to aff t the activity of the grub. Most grubs are very active unless badly in ted with the disease.

AUSAL ORGANISM.—Pure cultures of *M. nigrofaciens* show micrococci of varying izes, 0.9μ and 1.2μ to 1.4μ diameter with dividing forms; occur singly, in pairs, thi s (triangular), fours (tetrads or diamond shape), and clumps of more or less reg ar groupings, the individuals in a group arranged in a honeycomb-like order; ch s of more than three never observed. Gram-positive; stains well with anilin dy

rowth on agar abundant, beaded, flat, glistening, opaque, pale orange yellow, bu ous consistency, no odor. Cultures newly isolated are not pigmented and give on a moderate growth. Turbidity in broth, no ring or pellicle, no gas. Litmus mi is slightly reduced and acidified, no curd, yellowish deposit of bacterial cells. Ge in is liquefied; dextrose, lactose and saccharose not fermented. Moderate ind production; nitrates reduced.

METHODS OF INFECTION.—Sterilized soil was inoculated with a br h culture of the micrococcus and in the soil were placed apparently un fected larvæ, which were incised to imitate accidental abrasion. Cl racteristic lesions developed in two days at these points. Under na ral conditions these larvæ bite one another, especially if they are ve numerous in any one place; this may account for the rapid spread of e disease. *M. nigrofaciens* must be a common soil organism, especi y where this disease is common. Parasitic insects or fungi may als aid in making infection possible.

Excessively wet soil favors the progress of the disease. Larvæ of *Al hina nitida*, the southern June beetle, are susceptible to this infec tio but less so than the *Lachnosterna* spp. The American cockroach, *Pe planeta americana*, is also slightly susceptible, the infection limiting its to the legs.

M. nigrofaciens does not lend itself readily to the control of the wh e grubs on account of the limiting environmental conditions.

FLACHERIE, AN INFECTIOUS DISEASE OF SILK-WORMS

Streptococcus bombycis—Cohn

HISTORY AND DISTRIBUTION.—Flacherie appeared in the silk indus try as an epidemic at the end of the sixteenth century. It was again a ious epidemic about the year 1869 in the silk nurseries of southern

France. Later it was found in Italy and other neighboring count devoted to sericulture. In 1870 Pasteur recognized flacherie in s worm as a disease of the silk-worm distinct from pébrine.

Symptoms.—Diseased worms refuse to eat, become languid; a the fourth molt when they ordinarily climb up twigs and branches the purpose of pupating, instead of spinning their cocoons they stre out and remain motionless until death, or they may fall pendant, ha ing by their pseudofeet. Worms when dead appear so very life- that it is necessary to touch them in order to make sure that they not living. From this appearance comes one of the names of disease "morts-blancs."

After death they become soft in a short time and assume a blac color in twenty-four to forty-eight hours. The body is then fi with a brownish fluid swarming with bacteria. Hundreds of wo in this condition show no polyhedral bodies (characteristic of pébri A glance is all that is necessary to distinguish worms dead of flache

Causal Organism.—In the silk-worms as well as in culture *Streptococcus bo cis* forms short chains of small cocci, 0.89μ in diameter; the chains are from 5.01, 11.99μ long; stain well with anilin dyes and are Gram-positive.

In gelatin plate cultures, colonies are small, round, yellowish-gray, sharply toured, finely granulated interior, gelatin not liquefied. Subsurface colonies the same characteristics. Gelatin stab cultures are dull white, not liquefied. colonies are small, round with a slightly undulating contour, deep brown in c finely granulated, moist.

It is opalescent on glycerin agar and on ordinary agar when first isolated. broth at 37° a marked turbidity is manifested after twelve hours without floccule after long standing the broth becomes clear. Potato cultures show an iridesc which later becomes a light gray. *Strept. bombycis* develops in milk without curd it. It is a facultative anaerobe; the temperature optimum is 37° but it develops at 20° also. The streptococcus retains its vitality for a long time in culture. destroyed at 65°–70° in fifteen minutes.

Methods of Infection.—Infection of the silk-worm takes place means of food infected either with the excrement of sick individ or with the dust of infected silk-worm nurseries of the year preced

When silk-worms show all the symptoms of flacherie, if they dev into moths the eggs laid by these moths are always infected. If of the forms in which the silk-worm exists during its life cycle beco infected it is sure to die before the cycle is completed.

Certain environmental conditions favor the rapid developmen

flacherie; high humidity due to an approaching storm or to keeping the worms enclosed in a practically air-tight cage prevents the transpiration which is so necessary to the worm after the fourth molt. Too many worms together often favors the progress of the disease.

CONTROL.—Pasteur instituted the following means of producing healthy strains of the silk-worm; a small portion of the digestive cavity of moth was abstracted with a scalpel, mixed with a little water and examined microscopically. If the moths did not contain the characteristic microörganism, the strain they came from might unhesitatingly be considered as suitable for seeding. The flacherie organism was as easily recognized as the pébrine corpuscle, but the infection was more difficult to prevent on account of the environmental conditions above mentioned.

BACTERIAL DISEASE OF LOCUSTS

Bacillus acridiorum—d'Herelle

HISTORY AND DISTRIBUTION.—Tropical and subtropical countries covering more than half the earth's surface suffer periodically from plagues of locusts of different species. Famine and its attendant, epidemic disease, follow in their wake and decimate the regions invaded. A bacterial epizootic has become a natural means of control.

Bacillus acridiorum, the cause of the locust epizootic, was discovered in Mexico in the state of Yucatan by F. d'Herelle. In 1909 a certain mortality was noted among the swarms which arrived from the south of the state where they winter; the following year the epizootic was generalized and raged among a large number of bands; finally in 1911, all of the swarms which appeared were attacked, and in 1912, the locust invasion ceased. These particular locusts were the *Schistocerca paens*.

SYMPTOMS.—The locusts which are attacked by the natural disease, present symptoms which are identical with those which are experimentally inoculated or contaminated *per os*. After a time of incubation, which varies from one to forty-eight hours according to the virulence of the bacillus, the age and individual resistance of the insect, and the environment (temperature especially), at first the contents of the chylific stomach become liquefied and assume a dark color resembling coagulated blood. The locust ceases to eat, becomes flabby, jumps awkwardly and hides itself under tufts of herbage. The intestinal con-

tents next become liquefied; they are at first a clear yellow, later d
ening little by little until they are blackish in color. At this sta
slight pressure upon the abdomen causes the liquefied intestinal
tents to issue from the anus and the characteristic diarrhœa reveals i
on the vegetation which is fouled with the dejecta of the sick loc
Some hours afterward the locust falls upon its side and the legs m
spasmodically; the locust remains in this comatose state several
utes to several hours until death occurs. When the virulence of
coccobacillus is very high, very often the chylific stomach only pres
the characteristic blackening; death occurs before the intestinal
tents have undergone a modification. After death the insect putr
very rapidly and the tegument becomes dark.

The intestinal content of locusts attacked with this disease sh
microscopically practically a pure culture of this bacillus. The in
tinal contents of healthy locusts are poor in bacteria, sometimes se
ingly aseptic. Among the saprophytes found, the most common
motile, Gram-positive coccobacillus which causes death of locusts
injection but never by ingestion. It is distinguishable from the spe
coccobacillus by the disagreeable odor which it produces in the loc
or in culture media. Sometimes staphylococci are found, rarel
subtilis; only one saprophyte per hundred of the specific bacillus r
ers the isolation of the latter very simple.

All of the tissues of the locust are invaded by this bacillus as
been proved microscopically. A pure culture can be obtained from
blood at the same time that the intestinal contents are attacked,
B. acridiorum produces a veritable septicemia.

Causal Organism.—*B. acridiorum*, the causal organism of the Mexican l
epizootic is a short, slightly ovoid bacillus, decidedly polymorphous; in the
culture coccus forms of about 0.6μ are found beside of forms plainly bacilli, o
0.6μ×0.9μ to 1.5μ; actively motile possessing peritrichic flagella; Gram-negativ
stains readily with anilin dyes; the bacillus takes the stain most deeply at the
especially if Ziehl's carbol-fuchsin is used for one to two seconds.

Facultative anaerobe; cultures grow readily from 16° to 43° in all ordinary m
even in Raulin's medium. It develops very rapidly at 37° in broth, turbidity ap
ing at about the fourth hour. A delicate membrane is formed on broth which
only after three weeks, leaving a heavy sediment. Young agar colonies ar
cular and have a waxy appearance; they grow rapidly, being plainly visible
twelve hours; after eighteen hours, they are 2–3 mm. in diameter. Subsu
colonies are small, spherical, whitish and opaque. Gelatin is not liqu
Milk is coagulated and rendered strongly alkaline. Grows abundantly on p

aving a creamy appearance; the culture in the water at the bottom of the tube is dense that the liquid becomes sirupy and has a strong alkaline reaction. extrose, levulose, maltose and galactose are fermented; the inoculated medium ntaining one of these sugars becomes acid at first, then alkaline. This alkalinity due to the formation of ammonia. *B. acridiorum* has lived over two years in aled tubes.

Methods of Infection.—Natural. There are several natural ethods by which the epizootic is spread. Sick locusts or nymphs ave their infectious liquid dejecta on the vegetation, the other locusts t the contaminated herbage, contract the disease and in turn infect w plants thus continuing the cycle. With certain species of locusts, e *Schistocerca* for example, another very important mode of contagion ists: when one of their number becomes weak or where vegetation is arce both the nymphs and the adults eat one another.

At the time of depositing the eggs, if the female or even the male diseased, the eggs will be forcibly soiled with the liquid of the diarœa and the bacillus will be conserved up to the time of hatching upon e eggs or in the mucilaginous matter surrounding the eggs which the cust has provided for their protection.

A certain number of locusts among every swarm act as healthy arriers." Carriers among nymphs have never been found.

The period of life of the insect affects its resistance. The adult cust is individually much more susceptible than the nymph. The bits of life of each, however, have a great influence. The nymphs e continually in contact with the vegetation and with each other as ey march in very dense columns; they are endowed with a voracious petite and undergo in the short period of their larval life, five molts, hich are the periods of their least resistance. The winged locusts, the contrary, passing a large part of their life in the air, are only rely pressed one against the other, except, for example, when the ather is cold; they also eat much less than the nymph, thus the izootic will have a greater tendency to become generalized among the nds of nymphs than among the swarms of adult locusts.

The age of the nymph or locust influences its resistance; the young mphs have a maximum resistance, but this decreases gradually, aching its minimum at the time of the last molt; the adult locust has minimum of resistance at the egg-laying period.

The period of the molt is not a means of protection against this pe of disease, which is a generalized septicemia.

ARTIFICIAL.—The virulence of *B. acridiorum* decreases very rapidly in cultu and in order to obtain the desired destruction of locusts it is absolutely necessa to employ cultures of the highest possible virulence as an attenuated virus immuni the locust and renders it refractory to a culture of the highest virulence when appli later.

The virulence is increased by successive passages through locusts or nympl twelve series of passages are made using twelve locusts in each series. The c ture to be rejuvenated is mixed with a few cubic centimeters of sterile water or bro Injections are made with a syringe having a very fine sharp-pointed needle. T insect is seized with the left hand, the ventral portion toward the operator, and t needle of the syringe inserted between the second and third anterior abdomi segments at the point of intersection with one of the longitudinal ridges, horiz tally in the direction of the head to a depth of about 3 mm. for an adult insect little less for a nymph. The point of the needle should enter the abdominal cavi not merely pierce the tegument as in the latter case the effect would be nil. the needle is inserted too deep the internal organs will be injured. A very fi pointed bent pipette could be employed equally well. One or two drops of the em sion of the old culture are injected.

As soon as the locusts in the first series become sick or preferably are nea dead, press the abdomen between the fingers and collect in a watch glass the black liquid which issues from the anus. Inject a drop of this liquid into the abdomi cavity of the second series of locusts, following the same technic and observing same precautions as for those of the first series. These insects will die in a shor period of time. Obtain as previously, in a watch glass the intestinal liquid of th or four of the first dead locusts of the second series, dilute half with water and ste broth and inoculate the third series. To inoculate the fourth series, use the int tinal liquid of the first dead of the third series diluted to a third; a fifth series w the liquid diluted to a fourth and continue with the series in this way. It is exc tional that it will be necessary to proceed further than the twelve series. The vi lence of *B. acridiorum* is increased sufficiently if death occurs eight hours after inj tion. One-hundredth of a cubic centimeter of virus at its maximum virulence jected into a locust will cause the characteristic diarrhœa in two hours and de an hour later. This method of increasing the virulence takes five to six days this period of time has to be taken into consideration when it is necessary to emp the culture on a practical scale.

When the acridian to be infected belongs to a different species than that for wh the virulence of *B. acridiorum* has been previously augmented, a large number passages may be necessary as a culture virulent for one species may not be able infect another species. In one case fifty-two passages were necessary in order kill *Stauronautus maroccanas* (Algeria) in eight hours while for the same insect Cyprus only twelve passages were necessary. It is also desirable that the f few series consist of a large number of insects, as there will be apt to be so which will be more sensitive to the virus. Their natural resistance can be weake by fasting for several days before inoculation. The intestinal contents should be diluted until the virus will kill within fifteen hours.

When the virulence is sufficiently increased, the specific bacillus is isolated by rans of an agar slant or plate and cultivated for twenty-four to thirty-six hours a room temperature; it may then be isolated if the virus is to be conserved; if ired for direct infection experiments, it may be placed directly in broth. The th used is made as follows:

Wer.....	1,000 c.c.	Boil, alkalinize slightly and filter; place in bottles, plug with cotton, cover mouth and neck with parchment paper cap, and sterilize at 120° for thirty minutes.
Ptone.....	40 gr.	
S.....	5 gr.	
Gatin.....	30 gr.	
Dtrose.....	5 gr.	

T gelatin serves to fix the organisms in place when the culture is sprayed on the vetation, and on account of the dextrose the plants are greedily devoured by the locusts.

It is necessary to remember that the virulence of *B. acridiorum* lowers very rapidly in ulture and is attenuated likewise by re-transplantations, so that a broth culture s repared should be used within two or three days at the utmost. If the campaign a nst the locusts lasts for several months or the regions invaded are extensive, it ill be necessary to continue the series of passages during the campaign in order t ave on hand a virus of maximum efficiency. It is best to make two or three more p ages than necessary, rather than too few, for in the latter case the results of a w le campaign may be nullified.

The material necessary for a campaign against the locusts consists of a *new* sp y pump, preferably tinned inside, such as is used in spraying fruit trees, and the b les containing the pure broth culture of *B. acridiorum* at a maximum virulence. A ed spray pump should never be employed as it is practically impossible to free it om the antiseptic contained. The pure culture should be used as soon as it sh s turbidity. Broth cultures should never be used which have a putrefactive od.

In practice it is generally necessary to infect the greatest number of locusts in th largest possible number of bands in order to exterminate them with certainty in short a time as possible. The quantity of broth culture to be sprayed varies wi the area covered by the nymphs or locusts. One liter per hectare is sufficient in ll cases. For large areas, *e.g.*, 100–200 hectares, spray over twenty dif- fer t places, using one-half liter each time, taking in all ten liters. It is better to ray over a large area rather than all in one place, choosing places where the ny phs or locusts are in largest numbers, and always spraying the type of plants pr rred and in advance of where they are eating. Spraying should be done in the ear morning or preferably in the evening towards sunset. The heat and especially the right light of day rapidly attenuate the virulence of the bacillus. If necessary to ray in the middle of the day, shady spots should be chosen.

The virulence and vitality of *B. acridiorum* has been conserved in the dejecta and dried cadavers for seven months while in culture the virulence especially, is los apidly.

A very hard rain will inhibit the progress of an epizootic for several days. The rai washes the dejecta of the locusts from the contaminated vegetation, hindering

this mode of contagion; the epizootic little by little regains its normal activity. A rain of short duration, to the contrary, seems to favor the progress of the disea

A curious phenomenon takes place when a band of infected nymphs meet a r in the course of their route. On the near bank is found an actual heap of cadav on the opposite bank likewise but they are very much less in number. The i- zootic seems to be completely checked; it recommences only after several days w it takes its normal course. This is explained by the fact that all the nymph: l- ready badly diseased are not strong enough to make the necessary effort to cross stream and die without surmounting the obstacle; those which were only slig diseased could pass it but were so enfeebled by their effort that they died on opposite bank. Thus the colony which pursues its march is composed onl healthy insects and of several nymphs in which the infection has hardly begun.

The duration of an epizootic is impossible to predict for all species of insects under all conditions; as a general rule it will last several days, most often sev weeks, rarely several months. The duration of an epizootic, however, is of l importance; the object is to cause such a reduction in the number of the locusts these insects will cease to be a plague.

To spread the epizootic to great distances, care should be taken to infect winged adults. Some species of locusts are more sedentary than others, it fol that the more sedentary a species is, the more necessary to multiply the fo infection.

In order to ascertain whether the epizootic is progressing, gather one hun locusts from different parts of the swarm and by pressing their abdomens, see many show the characteristic diarrhœa. Those insects showing diarrhœa one will be dead the next.

Certain peculiarities were observed during the course of an epizootic. In sw infected a little while before egg-laying, numerous females lay eggs which n reach maturity; others never reach the laying stage and the eggs are transfo within the body to a blackish mass. Such bands were annihilated several afterward. In bands of nymphs infected several days before the last molt are f numerous abnormal adults with poorly developed wings only half their ordi length which prevent them from flying, and further a microscopic examinatic the genital organs shows complete atrophy.

Susceptible Insects.—I. Acridians.—*B. acridiorum* should pathogenic for all acridians. The following species are susceptible: *Schistocerca americana* (or *pallens*).—Natural epizootic in Yucata in 1908–1911, induced in the Argentine Republic in 1912.

Caloptenus sp?.—Epizootic induced in December 1912 in the re of Rio Negro, Argentine Republic.

Stauronautus maroccanus.—Epizootic induced in 1913 in Alg in the province of Oran, and in the isle of Cyprus.

Schistocerca paranensis is killed by *B. acridiorum*. (Argentine public.)

Zonocercus elegans, the so-called "elegant grasshopper" of South Africa, a non-migratory species, was used in inoculation experiments with this bacillus. It was concluded that this disease at best could be employed only as a supplementary measure in dealing with the invasion of these insects under conditions that prevail in South Africa.

The Philippine locust, *Pachytylus migratoroides*, has given negative results with *B. acridiorum*. (Mackie).

II. Ants.—A species of small ant near Paris was annihilated in 1911 by *B. acridiorum*.

Selenopsis gemminata, near Buenos Aires was annihilated in 1912. Several drops of the culture were placed in each ant hill.

Atta sexdens, a veritable plague in the tropical and sub-tropical countries was annihilated at Chaco and Tucuman after the virulence had been increased for this species of ant by many passages.

III. Caterpillars.—A field of cotton which was being ravaged by caterpillars was treated with *B. acridiorum*. Four days afterward all the caterpillars were dead while a neighboring field, treated simultaneously with Paris green, still contained many living caterpillars.

B. acridiorum does not attack the silk-worm, *Bombycis mori;* it kills the cockchafer, *Melolontha vulgaris*, by injection but not by ingestion. Birds and mammals in general are immune to this bacillus. One notable exception is the sewer rat which dies from generalized septicemia a few hours after injection. The rat was immune to cultures ingested.

Bacillary Septicemia of the Caterpillars of *Arctia caja* L.

Bacillus cajæ—Picard and Blanc*

History and Distribution.—In 1913 the vineyards of central France were almost completely destroyed by two diseases; one of these was a fungus disease caused by *Empusa aulicæ*, the other was a septicemia of bacillary origin.

Symptoms.—The caterpillars become flaccid and emit a nauseating odor; their digestive tube contains only a clear liquid free from all organisms. The blood contains a pure culture of a bacillus with which the disease has been produced artificially.

* Picard, F. and Blanc, G. R. On a bacillary septicemia of caterpillars of *Arctia caja* L. Compt. rend. acad. sci. 156, 1913, pp. 1334–1336.

CAUSAL ORGANISM.—*B. cajæ* is a slightly oval bacillus, about 1.5μ in length motile; Gram negative; stains deeply with crystal violet; treated by Pappenheim method it shows a characteristic bi-polar stain.

Broth cultures develop in twelve hours at 15°–35° with an optimum of 25°; from these the odor of H_2S is perceptible; in twenty-four hours broth cultures have a green fluorescence which is more marked at 25° than at 15° or at 35°. Grows rapidly on bot gelatin and agar showing a green fluorescence, the former is liquefied. Growth o potato is meager, showing only after forty-eight hours and producing no pigment.

METHODS OF INFECTION.—Artificial.—Caterpillars of *Arctia caj* inoculated in one of their feet by means of a fine needle dipped in virulent blood or in a broth culture, die regularly in three days at 15° manifesting in their blood swarms of the specific bacteria. If kept a 25° they die in twelve to twenty-four hours. The blood of the caterpillars kept at the latter temperature appears to be the more virulen

Caterpillars receiving several drops of culture by means of a pipette introduced into the pharynx, die in twelve hours at 25° with thei blood invaded by the bacteria. This suggests a possible practica application.

SUSCEPTIBLE INSECTS AND OTHER ANIMALS.—Caterpillars of *Porthesia chrysorrhea* are very sensitive to *B. cajæ* and die on inoculatio in twenty-four to forty-eight hours.

The following Coleoptera: *Hydrophilus pistaceus, Dyticus pisanus Cybister laterimarginalis, Colymbetes fuscus* are not killed by inoculation; nor are the following Hemiptera: *Notonecta glauca, Nipa cinerea Ranatra linearis.*

The white rat is not sensitive to intraperitoneal injection of 1 c.c. o a twenty-four-hour broth culture. The tree frog, *Hila arborea*, die by inoculation, with the same culture, into the lymphatic sacs i twenty-four to forty-eight hours with the blood invaded by numerou organisms. The blood of dying caterpillars is more virulent for th tree frog than broth cultures; 0.5 c.c. injected into the lymphati sacs causes the death of the batrachian in twelve hours with a intense bacillary septicemia.

B. cajæ seems to belong to the same group as d'Herelle's *B. acridiorum.* It is distinguished from it however by several characteristics both biological and pathological, being a parasite of the blood of th caterpillars whereas, according to d'Herelle, the site of affection in th diseased locusts is in the digestive tract.

GRAPHITOSIS*

Bacillus tracheitis or graphitosis—Krassilstschik

HISTORY AND DISTRIBUTION.—This disease together with a bacterial septicemia was noted among the *Lamellicornia* in 1893 in the southeast of Russia by Krassilstschik. He states that the larvæ from the *Lamellicornia* which formerly died *en masse* of muscardine,† die of this disease very seldom in recent years. Bacterial parasites seem to have replaced it in this part of Russia, and this is distinctly advantageous since the bacterial diseases are much more destructive than any of the species of muscardine.

SYMPTOMS.—At first the larva is entirely pure white, then several rings change to a bright yellow color, next to a yellow brown. Little by little this coloration extends over all the legs. Later on both sides of the larva, characteristically in the region of the spiracles and around them, the skin takes on a grayish hue which gradually deepens. The larva is generally living at this stage but appears to be diseased. This grayish coloration extends toward the back, and the anterior part of the larva then gradually becomes gray also. At this stage the larva is generally dead. After death, the gray color, spreading characteristically from the spiracles, deepens considerably, extending all over the skin, finally acquiring a tint resembling that of polished graphite, hence its name "graphitosis." This coloration is very characteristic for the larva which die of this disease; it is only very rarely that the cadaver is of a brownish shade.

When the infection first shows in the legs, the movements are not inhibited in any way, but when the graying around the spiracles sets in, the larva becomes comatose yet still responding to exterior excitations. It soon dies, retaining its characteristic curve but gradually becoming limp and soft and decreasing in size, length, etc.

CAUSAL ORGANISM.—The bacillus of graphitosis is from 2μ to 2.2μ long having a diameter of more than half its length; spores are produced; very motile, movements quick and rapid; occurs generally in pairs from 3.6μ to 4.6μ long; long filaments not produced; the longest do not exceed 7μ to 9μ which corresponds to two pairs of bacilli

*Bacillary Diseases of Lamellicornia.—In 1893 Krassilstschik described two bacterial diseases attacking the larvæ of the following insects: *Rhizotrogus solstitialis, Melolontha vulgaris, Anisoplia austriaca, crucifera, and fruticola, Cetonia* sp. and a larva belonging to the *Geotrupini* (*Lethrus?* sp.).

† Krassilstschik, I. Graphitosis and Septicemia of Insects. Memoires Soc. Zool. en France, t. vi, pp. 245–285, 1893.

end to end. Aerobic; shrinks perceptibly when treated with Gram's stain, almos to half its size. Stained bacilli show unstained spots (spores).

In plate cultures *B. tracheitis* develops into small circular colonies 0.25 to 0.7 mm. in diameter, which are covered with tubercles when the colony grows in gela tin. Gelatin colonies are finely granular of a deep brownish-yellow color, opaqu center surrounded by a transparent ring. Gelatin is liquefied in twenty-four t forty-eight hours. Gelatin stab cultures are typical, having a cup-shaped hollo funnel at the surface, a short empty stem; then the culture grows in the depth c the gelatin, liquefying it in the shape of a carrot, later becoming the shape of a inverted bottle; the culture is seen along the original path of the stab as a zigza line which later forms a compact cream-colored deposit as the gelatin become entirely liquefied; these cultures have the odor of the white of an egg. Brot cultures are clear the first twenty-four hours; after that they become turbid an a pellicle forms which thickens with age, sediment compact; cultures becom wholly transparent in four to six months.

Methods of Infection.—Washing the larvæ of the *Lamellicorni* with the natural virus of graphitosis kills 16.6 per cent to 100 per cen but an augmented virus gives 100 per cent mortality. The injectio under the skin of a very small drop of graphitosis blood is always fata for the larva even if the virus is weak.

B. tracheitis multiplies first in the blood system, then fills th Malpighian tubes, next characteristically in the trachea and then in th fatty bodies; the trachea becomes typically filled with black amorphou granules from whence the name of this organism; the fat cells are at tacked. The intima of the muscles, trachea and other organs is covere with bacilli, which however do not penetrate the organs themselves.

American Foul Brood

Bacillus larvæ—White*

History and Distribution.—American foul brood is the prevalen disease among bees in America and is distributed through all part of the United States, in Ontario, Canada, Switzerland, New Zealand Germany, England, and France and it is probable that it has a muc wider geographical distribution.

Symptoms.—American foul brood or simply "foul brood" usuall shows itself in the larva just about the time that the larva fills the cel and after it has ceased feeding and has begun pupation.

* White, G. F. The cause of American foul brood. Cir. 94. Bur. of Ent. U. S. Dept. of Ag 1907.

At this time it is sealed over in the comb. The first indication of t infection is a slight brownish discoloration and the loss of the well- nded appearance of the normal larva. At this stage the disease is n usually recognized by the beekeeper. The larva gradually sinks dwn in the cell and becomes darker in color, and the posterior end lies ainst the bottom of the cell. Frequently the segmentation of the lva is clearly marked. By the time it has partially dried down and bome quite dark brown (coffee colored) the most typical character- isc of this disease manifests itself. If a match stick or tooth-pick is ierted into the decaying mass and withdrawn the larval remains ad- he to it and are drawn out into a thread, which sometimes extends for seral inches before breaking.

This ropiness is the chief characteristic used by the beekeeper in dgnosing this disease. The larva continues to dry down and gradually les its ropiness until it finally becomes a mere scale on the lower side wll and base of the cell.

The scale formed by the dried-down larvæ adheres tightly to the c and can be removed with difficulty from the cell wall. The scales c best be observed when the comb is held with the top inclined to- wrd the observer so that a bright light strikes the lower side wall. A vy characteristic and usually penetrating odor is often noticeable in t decaying larvæ. This can perhaps best be likened to the odor of hted glue.

The majority of the larvæ which die of this disease are attacked aer being sealed in the cells. The cappings are often entirely removed b the bees, but when they are left they usually become sunken and fquently perforated. As the healthy brood emerges the comb shows t scattered sunken cappings covering dead larvæ, giving it a char- aeristic appearance.

Pupæ also may die of this disease, in which case they too, dry down, bome ropy, and have the characteristic odor and color. The tngue frequently adheres to the upper side wall and often remains there en after the pupa has dried down to a scale. Younger unsealed larvæ a sometimes affected. Usually the disease attacks only worker bods, but occasional cases are found in which queen and drone broods a diseased. It is not certain that race of bees, season, or climate lve any effect on the virulence of this disease, except that in warmer

climates where the breeding season is prolonged, the rapidity of dev. tation is more marked.

CAUSAL ORGANISM.—*Bacillus larvæ*, a motile, spore-producing bacillus is etiological factor in American foul brood. It is cultivated with difficulty, grow best on media made as the ordinary laboratory media, substituting bee larvæ meat. On these media it produces a large number of giant whips which persist fo long time. These giant whips are also found in decaying larvæ which are dead fr American foul brood experimentally produced by feeding pure cultures of *B. lar B. larvæ* and its spores are killed at 90°–100° in ten minutes, and at 100° in less th five minutes.

METHODS OF INFECTION.—Artificial.—American foul brood can communicated by feeding to a healthy colony the scales from com which had contained brood affected with American foul brood; likew when these scales are placed in ordinary meat broth, incubated twent four hours and then heated to 65° for twenty minutes; infection in th case is due to the presence of spores. Pure cultures of *B. larvæ* mix with sterile sugar sirup and fed to healthy colonies produces the disea within three weeks. *B. larvæ* can be obtained in pure culture fro such diseased larvæ.

B. larvæ may be obtained in large quantities suitable for expe mental inoculation by diluting and filtering the crushed bodies bee larvæ through a Berkefeld or other fine filter.

CONTROL.—The treatment of an infectious bee disease consis primarily in the elimination or the removal of the cause of the disea Effort is not made to save the larvæ already dead or dying, but to st further devastation by removing all material capable of transmitti the cause of the trouble. The swarm is transferred from the infect hive to a clean disinfected hive; the infected combs from the old hi should either be burned, melted, or boiled thoroughly before the wax fit for use again. The honey taken from the infected hive should buried or at least removed so that no bees can use it for food. Th treatment may have to be repeated before the disease is under contr Brood from badly diseased colonies should be burned, buried or othe wise destroyed at once. Combs even if they appear white and cle should be melted. Infected hives should be burned over inside wi a gasoline or oil torch.

Septicemia of the Cockchafer, *Melolontha vulgaris*

Bacillus melolonthæ—Chatton*

History.—In May 1912, while studying the effect of d'Herelle's . *acridiorum* on the cockchafer, Chatton noticed that the cockchafers ere dying from a spontaneous septicemia; this he found later was due) a coccobacillus which he named *B. melolonthæ.*

Symptoms.—No symptoms are noted.

Causal Organism.—*B. melolonthæ* resembles *B. acridiorum* of d'Herelle with the ception of the following characteristics: the bacillus is longer, and in agar culture oduces a green fluorescence in five to six days. It is distinguished from the bacillus d'Herelle in addition by its pathogenic action on the silk-worm, *Bombycis mori.*

Methods of Infection.—Injected into the general cavity, *B. elolonthæ* kills the cockchafer in twelve to thirty-six hours, and where s virulence has been augmented by several passages through this sect, always in less than twenty-four hours, but *per os* it is as active as *B. acridiorum.* Seventy-five per cent. of healthy cock-afers show the presence of *B. melolonthæ* in their digestive tube, metimes in massive culture. This is always the case with cock-afers affected with septicemia.

This blood disease seems to be of intestinal origin however, as with e locust. *B. melolonthæ*, a common parasite of the intestine of the ckchafer passes into the general cavity only under special conditions t unknown. When this organism is removed from the intestine and ected into the general cavity, septicemia is produced.

It is as virulent for the silk-worm by injection as for the cockchafer, d as inactive by ingestion.

European Foul Brood

Bacillus pluton—White†

History and Distribution.—This type of foul brood, sometimes own as "black brood," or "New York bee disease" is not nearly as le spread in the United States as is American foul brood, but in cer-

* Chatton, E. Spontaneous septicemia in the cockchafer and the silk worm due to cocco-lli. Compt. rend., acad. sci. 156, 1913, pp. 1707-1709.

† White G. P. The cause of European foul brood. Cir. 157, B. of Ent. U. S. Dept. of A 1912.

tain parts of the country it has caused enormous losses. It is spread over England, Germany, Switzerland and other parts of Europe and has been noted many times during the last decade.

Symptoms.—The presence of disease can usually be detected in an experimental colony during the week that feeding is begun. The first indication of it may be that only a portion of a larva is seen in a cell the remaining portion having been removed by the bees. Aside from an observation of this kind, the earliest indication one gets from the macroscopic examination is that sick larvæ are found among the uncapped brood.

Sick larvæ manifest certain symptoms during the course of the disease by which its presence can be diagnosed while the larvæ are still alive. The length of time that a developing bee is sick of European foul brood is variable. In general, the three days just preceding the time when a larva would ordinarily be capped, is the most favorable period for making a diagnosis from the gross examination alone. Healthy larvæ at a certain age when slightly magnified show a peristalsis-like motion of their bodies, but larvæ of this same age when sick frequently exhibit a marked peristalsis which can easily be seen with the unaided eye. Diseased larvæ may show a yellowish tint or appear transparent instead of the glistening white or bluish white of healthy larvæ.

Another symptom often serves for diagnosis. In a healthy larva a pollen-colored mass is frequently plainly seen through the transparent area along the dorsal median line. If this intestinal mass appears white or yellowish white, the presence of European foul brood is almost certain. This may be often more plainly observed if the larva is removed from the cell with forceps.

European foul brood may be positively diagnosed in living larvæ of a favorable age and condition by the following method; Remove the larva to be tested from the cell and place it upon glass, preferably with a dark background; with a dissecting needle in each hand and with their points near together, pierce with both needles so as to tear the body wall crosswise, and continue to separate the two portions of the larva. If the larva is diseased, and one is successful, it will be found that the intestinal content will be stripped from and pulled out of the posterior and blind end of the canal. The intestinal content of healthy living larvæ cannot be removed in this way. The force which is ap-

ped in pulling the mass from the intestine frequently causes the typ-
il transparent, mucus-like substance surrounding the central mass to
setch and the enclosed whitish substance to break into segments;
ts appearance is very characteristic.

If the disease is more advanced, a portion of the intestinal content
ny flow out in the form of a sac, the wall of which is very easily bro-
k. When broken the content of this sac-like structure will flow out
a rather thin whitish or yellowish white fluid containing small whitish
gnules that vary in size. If the disease is far advanced and the larva
pbably dead, the enveloping substance of the intestinal content is
seasily broken that often only the whitish or yellowish-white fluid
flvs from the ruptured wall of the larva.

Dying larvæ diseased with European foul brood frequently show
t segments of the body marked off less distinctly than living healthy
lavæ.

Causal Organism.—*B. pluton*, the organism of European foul brood, is a small, n-spore forming organism, sharply pointed at one or both ends, about 1μ long and lethan 0.5μ in breadth, on the average; occurs frequently in pairs; single individuals v very markedly in size and shape.

This organism has never been cultivated, but sections of larvæ in various stages o he disease show *B. pluton* to be the first invader of healthy larvæ. *B. pluton* is ea y killed between 60° and 65° in ten minutes.

Methods of Infection and Control.—These are essentially the sa e as those for American foulbrood.

Bacterial Septicemia of Larvæ of the Lamellicornæ

Bacillus septicus insectorum—Krassilstschik

History and Distribution.—This disease occurred separately a together with graphitosis previously described.

Symptoms.—The septicemia produced in larvæ of the Lamelli-
cnæ by *B. septicus insectorum* is characterized by a uniform browning o he body of the larvæ. As death approaches, the larva shrivels up a when dead is about half its natural size and is of a deep brown color. D ing the progress of the disease, the larva ejects a very black, abund-
a viscous, semifluid substance from the anus which soils the ex-
tr nity of the abdomen.

CAUSAL ORGANISM.—This bacillus is 1.2μ to 1.8μ long and from 0.6μ to 0.9μ in c ameter; often in pairs; long filaments not formed. Spores are characteristically di lospores although isolated ones occur not infrequently. It shrinks very little wh stained by Gram's method.

Gelatin is liquefied; subsurface colonies are decidedly lemon shaped, yellowi brown, finely granular, surface of colony typically curled. Surface colonies are co centrically three-ringed, the interior opaque, the second ring more transparent, t third very thin and finely granular. Saccate liquefaction in gelatin stab; the gelat is blackened and has a very disagreeable odor. Spores are found in the sediment the bottom of the tube. Broth is made turbid in eighteen to twenty hours, pellicle, bad odor.

METHODS OF INFECTION.—Healthy larvæ inoculated with *B. se ticus insectorum* by placing cotton saturated with a broth culture on wound, died in most cases with typical symptoms.

The sole habitat of these microbes before death is in the bloo system.

BACTERIAL DISEASE OF THE GUT-EPITHELIUM OF *Arenicola ecaudata*, the Lug-Worm

Bacterium arenicolæ.—Fantham and Porter.*

HISTORY AND DISTRIBUTION.—This bacillus was found in the lum of the gut and within the intestinal epithelium of specimens of *Aren cola ecaudata* obtained from Plymouth, England. This disease is not frequent occurrence.

SYMPTOMS.—No external symptoms are noted. Lesions are pr duced in the epithelium, the cells undergoing degeneration, perha shortening the life of the lug-worm. *Bact. arenicolæ* seems to be co fined chiefly to the ciliated tracts, as was determined by microscopic examination of sections.

METHODS OF INFECTION.—No methods of infection are note From the type of the disease, however, infection *per os* is suggested.

CAUSAL ORGANISM.—*Bacterium arenicolæ** averages about 11μ long and broad. Extreme individuals measure 7μ to 17μ long by 0.7μ to 1.3μ broad; some the larger forms are slightly sinuous in outline; no flagella; chromatophile granu determined by staining with iron-hematoxylin, are present, often in considera numbers, scattered through the cell; these granules are often concentrated ir transverse bars both of which in some specimens are refractile. The cytopla stains with difficulty with plasma stains. Division is transverse. One termi

* Fantham, F. B. and Porter, A. *Bacillus arenicolæ*, n. sp., a pathogenic bacterium from gut-epithelium of *Arenicola ecaudata*. Cent. f. Bakt. I., Orig. 52, 1909, 329–334.

spore is formed which does not cause the enlargement of the rod to any extent. Neucultural characteristics are given.

IMPORTANCE.—This disease is of no special economic importance.

Sac Brood, a Disease of Bees

Filterable virus—White*

HISTORY AND DISTRIBUTION.—A disease which was similar to, but was not foul brood was noted in 1881 by Doolittle in America, by Jones of Canada in 1883, and by Simmins of England in 1887. The larvæ were found to die here and there throughout the brood comb; the disease would disappear entirely or it would reappear the next season; the bees would frequently remove the dead brood and no further trouble would ensue. Simmins found no microscopic evidence of disease in these larvæ. In 1892 an editorial in one of the bee journals stated that dead brood had been encountered which did not seem to be infectious and which lacked two decisive symptoms of the real foul brood, *i.e.*, the ropiness and the "glue-pot" odor. In 1902 G. F. White of the U. S. Dept. of Agriculture began the study of this diseased brood. This disease was described in Switzerland in 1906 and later in 1910.

The name "sac brood" comes from the fact that many larvæ dead of the disease can be removed from the cell without rupturing their body wall. When thus removed they have the appearance of a small enclosed sac.

SYMPTOMS.—The strength of a colony in which sac brood is present is frequently not noticeably diminished. When the brood is badly infected, however, the colony naturally becomes appreciably weakened thereby. The brood dies after the time of capping. The dead larvæ are therefore, almost always found extended lengthwise in the cell and lying with the dorsal side against the lower wall. It is not unusual to find many larvæ dead of this disease in uncapped cells. Such brood, however, had been uncapped by the bees after it died. In this disease the cappings are frequently punctured by the bees. Occasionally a capping has a hole through it, indicating that the capping itself had never been completed. A larva dead of this disease loses its normal

*White, G. F. Sacbrood. Cir. 169, B. of Ent., U. S. Dept. Agr., 1913.

color and assumes at first a slightly yellowish tint. "Brown" is the most characteristic appearance assumed by the larva during its decay. Various shades are observed. The term "gray" might sometimes appropriately be used to designate it. The form of the larva dead of this disease changes much less than it does in foul brood. The body wall is not easily broken, as a rule. On this account, often the entire larva can be removed from the cell intact. The content of this sac of larva is more or less watery. The head end is usually turned markedly upward. The dried larva or scale is easily removed from the lower side wall. There is practically no odor to the brood combs.

CAUSAL ORGANISM.—No microörganisms have been found either culturally or microscopically. However, experimental evidence shows that the etiological factor is a filterable virus.

METHODS OF INFECTION.—Larvæ, sick and dead of sac brood were picked from the combs, crushed and diluted with sterile water. The suspension was filtered by means of the Berkefeld filter. This filtrate was fed in sirup to healthy colonies with the result that the typical symptoms of the disease were produced in all cases.

The virus is killed by heating at 60° for ten minutes.

WILT DISEASE OR FLACHERIE OF THE GIPSY MOTH CATERPILLAR, *Porthetria dispar* L.

Filterable virus—Glaser*

HISTORY AND DISTRIBUTION.—There is no account of the occurrence of wilt in America prior to 1900. This disease may have been introduced on trees or shrubs imported from Europe, in which country "Wipfelkrankheit," a wilt disease of the European nun-moth caterpillars, *Psilura monacha*, exists. In Europe flacherie has become the "guardian angel" of the central European forests.

In the United States there is every reason to suppose that the wilt is distributed over the entire territory infested by the gipsy moth, a territory of about 4,850 square miles (1915) extending over various parts of Maine, New Hampshire, Massachusetts and Rhode Island.

SYMPTOMS.—The symptoms of the wilt disease of the gipsy moth

* Glaser, R. W. and Chapman, J. W. The Wilt Disease of the Gipsy Moth Caterpillar. Jour. Econ. Entomol., 6, 1913, pp. 479–488.
Reiff, W. The Wilt Disease, or Flacherie, of the Gypsy Moth, 1911.

aterpillars are those of flacherie of the silk-worm. They soon stop ating, become languid, usually crawl up on some object where they emain motionless. In a few hours there drops from the mouth and nus a dirty, blackish, foul-smelling liquid; they become more and more accid, one leg after another looses its support and finally the caterillar reduced to a black skin is found hanging limply to tree trunks nd limbs, still holding on with one or two of its false feet or with the nal claspers. After death their body tissues become degenerated so apidly that it is impossible to handle them; a slight touch breaks the kin and a thin dark, offensive-smelling liquid flows out, consequently ney can never be used for histological work.

Causal Organism.—A filterable virus seems to be responsible for he death of these caterpillars. It is filtered with difficulty, however. Bacteria are not responsible for this disease. Minute dancing granules re observed in the Berkefeld filtrate, which may be etiologically sigificant. No bacteria or polyhedral bodies are observed in the filtrate.

Methods of Infection.—Infection naturally takes place through he mouth by means of the food. Predisposition to the disease is seured by giving the caterpillars food which has been placed in water nd renewed only every three or four days. This causes an increase in he acidity of the leaves which in turn decreases the alkalinity of the aterpillar's digestive fluid. Before the visible outbreak of flacherie, as n early symptom, a characteristic sweet odor is recognized in the reeding cages which resembles that of withered lilac blossoms somewhat. Whenever this odor is noticeable, flacherie soon makes its ppearance, and as it progresses the odor increases proportionately. (Fischer).

Lack of food, which is necessarily brought about by the caterpillars themselves, causes them to lose their vitality, thus producing a greater susceptibility to the disease. Defoliation also exposes them to the sun's rays which have the effect of converting the chronic into he acute form of wilt. In lightly infested woodland this does not happen as the caterpillars can always find shade. Flacherie, however, seems to be influenced by climate and weather conditions ess than any other caterpillar disease.

Wilt is always prevalent among the older caterpillars; young caterpillars often live several days before succumbing to the disease. Female caterpillars always succumb more readily to the wilt disease than the

male; this may perhaps be due to the fact that they require a longe time to mature than the male. Diseased females deposit egg cluste reduced in size, which contain usually, embryos, incompletely or nc at all developed. In this case there are always found undeposite eggs within the body of the female, which never occurs with health moths. Genetic immunity of certain individuals is probable. Sul lethal doses of the virulent filtrate may produce active immunizatio Although probable, there is yet no definite evidence that wilt is trans mitted from one generation to another.

Pathology of Wilt.—When a caterpillar dies of wilt, all of it tissues are in a state of disintegration. The intestine is the last in ternal organ to disintegrate. A smear of the brown liquid from a dea caterpillar examined microscopically with a high power lens will b found to contain, besides the elements of disorganized tissues, myriad of highly refractive polyhedral bodies of various sizes. The averag polyhedron measures from 1μ to 6μ in diameter and is never regular a are the silk-worm polyhedra. The significance of these bodies is no known. However, they are believed to be reaction bodies belonging t the highly differentiated albumins, the nucleoproteids. They may b stages of the filterable virus but no evidence has been brought forwar to substantiate this view. It has been determined however, that n diagnosis of wilt is valid unless polyhedra are demonstrate microscopically.

The "Wipfelkrankheit" of the nun-moth in Germany is essentiall the same disease as that of the gipsy moth in the United States (Es cherich and Miyajima.)

Pébrine, an Infectious Disease of the Silk-worm, *Bombycis mori*

Nosema bombycis

History and Distribution.—About the year 1853, anxious atten tion began to be given in the southern part of France to the ravages o a disease among silk-worms which from its alarming progress, threatene to issue in national disaster. Symptoms of this disease had been note as early as 1845. It finally became necessary to import seed (technica term for eggs) for continuing the culture of the silk-worms. This wa procured first from Lombardy, but after one successful year the sam

appointments occurred. Then Italy was attacked, also Spain and stria; later seed was procured from Greece, Turkey, the Caucasus, t to no avail; China itself was attacked and in 1864, healthy seed uld be obtained only from Japan.

This disease, characterized by dark spots on the silk-worms, was led pébrine, from the patois word pébré (pepper), the name given to by de Quatrefages on account of the resemblance of these spots to pper grains.

Symptoms.—As above mentioned, one of the symptoms of pébrine he manifestation of dark spots in the skin of the larvæ; some worms guish on the frames in their earliest days, others in the second ge only, some pass through the third and fourth molts, climb the g and spin their cocoons. The chrysalis becomes a moth, but the th shows signs of disease in its deformed antennæ and withered legs; wings seemed singed. Eggs from these moths were inevitably unccessful the following year. Thus, in the same nursery in the course the two months that it takes a larva to become a moth, the pébrine ease was alternately sudden or insidious; it burst out or disappeared, hid itself within the chrysalis and reappeared in the moth or the eggs a moth which had seemed sound.

Causal Organism.—The causal organism for this disease is microspic but nothing further of its nature is known.

In the worms suffering from pébrine, corpuscles or polyhedral dies, first noted by Pasteur, are found in all tissues and all fluids of body, even in the material from which the silk is made; naturally y are also found in the dejecta of the worms. These same bodies found in and on the infected eggs, pupæ and moths and in innumerae quantities in the dust of the infected nurseries; they are easily cognized microscopically.

Although these polyhedral bodies are neither known to be the etiical factor, nor the effect of the disease, elimination of all eggs, ntaining these bodies results in its suppression.

It is generally accepted, however, that *Nosema bombycis* is the cause of pébrine. spores find their way from the caterpillar by means of the dejecta or through the ntegration of dead forms to other silk-worms. Some of the parasites find their into the ovary, produce spores, pass through the pupal and imaginal stages of host into the next generation of silk-worms. The spores are often regarded as rine-corpuscles.

METHODS OF INFECTION.—A very common method of infection is due to the habits of the silk-worms crawling over one another. When a worm moves across a diseased worm, its claws cut through the tegument and become contaminated; in its progress it inoculates other worms by means of its soiled fangs. The greatest source of contagion, however, is the excreta which fall on the food of the worms. Luckily this infectious material on being exposed to light and air, becomes rapidly attenuated. However, the causal organism is not so attenuated when within the egg; it passes the winter in a latent state and develops along with the worm, multiplying within its body and altering more or less profoundly the conditions of existence.

CONTROL.—If moths are not seriously diseased, their eggs will always furnish several healthy larvæ and if these are isolated as soon as they hatch out and are kept and bred under sanitary conditions a race of worms free from corpuscles can soon be obtained. This has been found to be the most effective method of combating pébrine. Excessive heat saps the vitality of the silk-worm and makes it ready prey to disease. Open air cages resulted in much hardier, more active worms.

DIVISION VIII

Microbiology of Diseases of Man and Domestic Animals

CHAPTER I*

METHODS AND CHANNELS OF INFECTION

Infection Defined

The term infection implies the entrance of animal or vegetable rganisms into the body of another animal or plant, their multiplica-ion and their injury to that body. In most instances the organisms nter the tissues of the animal or plant body, although this is not true n every case of infection. It is possible in certain instances to produce he symptoms of an infection by introducing into the body the hemical products elaborated by some pathogenic organisms. For xample, the injection of tetanus toxin into the body causes the typical ymptoms of tetanus to result. Tetanus toxin is made by growing *. tetani* in beef broth under anaerobic conditions and filtering out the acteria by passing through porcelain filters. These chemical prod-cts do not occur naturally unassociated with the pathogenic rganisms and therefore they do not produce infections when artifi-ially injected in the usual sense.

The disease-producing organisms with which we will especially oncern ourselves in the subsequent discussion are those which are very inute in size and are of three kinds: first, bacteria; second, protozoa; nd third, ultramicroscopic microörganisms or viruses.

It is essential to have clearly in mind what is meant by an infectious isease and a contagious disease before entering into any detailed dis-ussion, although some authorities attempt to make no distinction. n *infectious disease* is any disease produced in the plant or nimal body which is due to a foreign animal or plant organism. The ame is applied to the nature of the cause of the disease. A *contagious*

*Prepared by E. F. McCampbell.

disease is an infectious disease which is transmitted from one individu to another by contact. The term refers to the method of transmissio rather than to the cause of the disease. It is possible that certain conta gious diseases may be transmitted by indirect contact or by the agenc of fomites but many authorities now hold the view that these facto are non-essential and that most contagious diseases are transmitted b direct contact.

Microörganisms of Disease Considered and Classified

Pathogenic Bacteria.—Bacteria which produce disease are know as *pathogenic bacteria*. Of the many thousand species of bacteria onl a comparatively few species have anything to do with the disease processes in the plant or animal body. Those bacteria which a capable of growing in the body of animal or plant may be designated a parasitic bacteria. Some bacteria can grow only in the animal or plan body and do not exist for any period of time outside of it. They a known as *obligate parasites*. There are others which may produc disease in the animal or plant body which can grow and reproduc outside the body. They are known as *facultative saprophytes*. Ther are still other bacteria which ordinarily live outside the animal an plant body and which exist largely upon dead organic material, whic when taken into the body occasionally produces disease processe They are called *facultative parasites*. As an example of an obligat parasite the *Bact. lepræ* of leprosy may be cited, although in th instance certain observers have claimed to have cultivated the bacill in pure culture. However, the results are not in any sense uniform Improved bacteriological technic has made possible the cultivation of large number of bacteria which heretofore were regarded as obligat parasites. As examples of facultative saprophytes the *B. typhosus* typhoid fever and the *Msp. comma* of cholera may be mentione As examples of facultative parasites *B. tetani* of tetanus and *Bac welchii* of gaseous gangrene may be mentioned.

Pathogenic Protozoa.—There are several infectious diseases i man and animals which are caused by pathogenic protozoa. Amon the common diseases due to protozoa there may be mentioned malari syphilis, rabies (the nature of the organisms involved in syphilis, an rabies is not well understood however), amœbic dysentery, Texas feve

nfectious jaundice of dogs, and the various trypanosome infections, uch as sleeping sickness, nagana, dourine, and mal de caderas. It is ifficult to artificially cultivate the pathogenic protozoa outside the nimal body in pure culture. The *Trypanosoma brucei* of nagana and he *Trypanosoma lewisi* of the rat have been cultivated. The *Entamœba coli* and the *Entamœba tetragena* of dysentery, the various ypes of the *Plasmodium malariæ*, and the *Treponema pallidum* of yphilis have also been cultivated, and it is stated that under certain onditions the *Piroplasma bigeminum* of Texas fever may be artificially rown.

Ultramicroscopic Microörganisms or Viruses.—There are some nfectious diseases the causes of which have never been discovered. The infectious agents in most instances cannot be cultivated and annot be stained by the ordinary bacteriological methods. The resence of ultramicroscopic organisms has been demonstrated in everal ways. For example, when the ordinary bacterial culture is un through a fine porcelain filter, the filtrate contains no microörgan-sms and consequently when inoculated into animals is non-infectious; lthough if soluble toxins be present there may be evidences of an ntoxication. When the viruses or the infected body fluids of men or nimals suffering from the diseases mentioned below are passed through fine porcelain filter the filtrate remains infectious, therefore demon-trating that the viruses or microörganisms are filterable and are prob-bly so small that they cannot be seen. Examples of diseases due to gents belonging to this class are as follows: hog cholera, yellow fever, oot-and-mouth disease, rinderpest, epithelioma contagiosum of fowls, hicken typhus, horse sickness, acute poliomyelitis, etc. There are everal infectious diseases of unknown cause, the viruses of which are ot filterable; for example, smallpox, cowpox and vaccinia, typhus fever nd Rocky Mountain spotted fever. There are still other diseases of nknown cause about which nothing is known regarding the filterability f the etiological agents of the disease. Scarlet fever, chickenpox and easles belong to this class. These diseases can be inoculated into nimals only with great difficulty and the virus cannot be cultivated r secured in sufficient quantities from the experimental animals for tudy. A possible explanation of some of these diseases of unknown ause may be found in the proposition that two microörganisms may ach produce non-toxic substances, and that when these non-toxic

substances come together, a toxic substance may be produced. This condition of affairs might explain certain infectious diseases in which microörganisms are known to occur, and in which they cannot be directly connected with the disease as causative factors. For example, the *Strept. pyogenes* very frequently occurs in both scarlet fever and smallpox. It has been shown absolutely that this organism is not the cause of these diseases, but there is a remote possibility that it may act in the so-called associative relation with some other microörganism or virus, as mentioned above, and produce the typical symptoms of these diseases. It has been recently stated that scarlet fever is due to a filterable virus but there is every reason to believe that the occurrence of the *Strept. pyogenes* materially changes the character of the infection and makes it more severe. The associative relationship of infectious organisms is probably not the logical explanation for all infections of this character. It might be mentioned in this connection that the view is held by some investigators that some of the infectious diseases of unknown etiology are due to enzymes and that a so-called autocatalysis explains the seeming reproduction in the body of the viruses. This theory is, however, without substantial proof.

The Distribution of Pathogenic Microbic Agents in Nature

The causal microörganisms of most of our infectious diseases are found principally in the bodies of diseased man and animals. There are some exceptions to their being found only in the bodies of the diseased. Notable examples are found among certain of the wild animals such as the brush-buck, wildebeast and others which serve as reservoirs for the microörganisms of some of the most fatal of protozoal diseases. These animals seem to be naturally immune. Various insects which are factors in the transmission of certain infectious diseases do not suffer from these diseases in any form and are naturally immune. The most common source, however, is the diseased animal or human body. There is no doubt, for example, that the natural habitat of the *Bact. diphtheriæ* is in the throat and nasal passages of persons suffering from or convalescing from diphtheria. Occasionally these bacteria are also found in the nasal passages and throats of persons who have never had diphtheria. The *B. typhosus* of typhoid fever also has its natural abode in the intestinal tract of persons suffering from or convalescing from the fever. The same is true with the majority of the causal microörgan-

ias. There are some microbic agents, however, which exist in the soil t probably do not undergo multiplication such as the *B. tetani* of tanus or lockjaw, *Bact. welchii* of emphysematous or gaseous gangrene, al the *B. botulinus* of meat poisoning. These bacteria sometimes st in the intestinal tracts of animals such as the horse and in all prob- ality their occurrence in the soil is due to their deposition in manure.

Te Occurrence of Pathogenic Microbic Agents Upon and In the Bodies of Healthy Animals and Man

The exposure to the air of the external surfaces of the body, of ourse, makes it especially easy for microörganisms to collect upon tm. The large percentage of the microörganisms which collect on t external surfaces are non-pathogenic but there are frequently dis- ee-producing ones among them. The various varieties of the *M. pgenes* are almost universally present on the skin and also on the eosed mucous membranes. *Strept. pyogenes*, *Bact. influenza*, *Bact. berculosis*, *M. intracellularis var. meningitidis*, *Strept. pneumoniæ*, *Bct. diphtheriæ* and many other species may be present. The mouth al nose are excellent places for microörganisms to collect and excellent f their growth as the requisite conditions such as food, heat and nisture are present. It has been stated on competent authority that a the species of bacteria which have been described as occurring in vious parts of the body have also been found in the mouth. These bteria do not necessarily produce disease or injure the body unless t vitality is lowered and they enter into the tissues. They feed upon t desquamating cells and the excretions. It is exceedingly interest- i to note that *Bact. tuberculosis* and *Bact. diphtheriæ*, as before stated, hve been found in the nose of persons who have never had these dis- ees. These bacteria have also been shown to be virulent and doubtedly such persons are extremely dangerous to other more sceptible persons. It is also frequently noted that pathogens are fnd in the bodies of persons after they have recovered from the dis- ee and that these individuals disseminate the microörganisms and iect non-immune individuals. This may be the case in diphtheria, thoid, Asiatic cholera and dysentery "bacillus carriers."

In regard to the occurrence of microbic agents in the internal organs the body the following may be said. For a long time it was claimed t the internal organs of man and animals were sterile. Neisser is

one authority for the statement that the internal organs of healtl animals are sterile. This has been shown not to be the case universall Experiments have shown that fifty per cent of the internal organs rabbits, guinea-pigs, cats, dogs, mice, horses and cattle are not steri *Bact. tuberculosis* has been found in absolutely normal human a bovine lymph glands. The various pus-producing micrococci ha been frequently found in the spleen, kidney, liver, etc. Perhaps t commonest group of bacteria to be isolated from the internal organs a the intestinal forms. It has been demonstrated that intestinal mic örganisms invade the tissues with surprising rapidity when for a reason the resistance of the body is lowered. It has been noted al that there are more bacteria in the internal organs of animals whi have been fasted than in those which have been fed. Peristaltic acti and the diffusion of food through the intestinal wall may be influenci factors. The fact that the internal organs are not sterile in every ca is important as it may account for the so-called autogenic infectio

The Manner Infectious Agents Enter the Body and The Source

Air-borne Infections.—The causal microörganisms of infectio diseases are frequently excreted from the body of the diseased individu and are deposited on the clothing, furnishings, on the floors and wal or on the ground. These microörganisms probably do not prolifera except in rare instances, but frequently remain virulent for a shc period of time and are capable of being carried through the air for shc distances, producing in certain instances disease in other individua There is no doubt that in diseases such as smallpox, measles, scarl fever and other acute exanthematous diseases together with su diseases as plague and diphtheria, that the infectious agents may carried through the air after having been deposited on clothing a furnishings. However, recent investigations have shown that th method of transferring infection is comparatively rare and that mo infections are transmitted by direct contact.

In the beginning it was supposed that the only way that bacte could be carried in the air was after having been dried on particles dust and carried by currents of air. This, however, has been sho not always to be the case and we now know that infectious micr

ganisms may be carried on small particles or droplets of sputum or oisture. These two types of aerial infection are known, respectively, dust and droplet infection.

Dust Infection.—Infectious microörganisms to remain virulent and able to produce infection must be able to successfully resist drying ter being affixed to particles of dust. After being dried the particles e frequently moved and whirled about by air currents. The larger rticles of material quickly settle down but the small, almost invisible eces of dried material may remain suspended for three or four hours. is these small particles which are usually inhaled or deposited on the in and mucous membranes of normal individuals that produce in-ctions providing the microörganisms have not been killed by drying exposure to sunlight. *Bact. tuberculosis* is sometimes carried in this ay as well as certain other pathogens. The fact that small-pox virus mains active after drying indicates, at least, that dust containing may be infectious. The extent of such dissemination is quite limited.

Droplet Infections.—It has been demonstrated that during the pro-sses of talking, coughing, and sneezing, small bubbles or droplets of utum are thrown out into the air. These particles remain suspended r some time and may be inhaled or deposited elsewhere. It is sur-ising the distance that these small particles may be carried. It is ated that they are frequently thrown out thirty feet or more. It has en shown that *Bact. tuberculosis* is rarely thrown out over four or five et by the cough of the tuberculous individual. It should be re-embered that these bacteria will remain alive two to three weeks hen in the dark but that they live only a few hours when exposed the sunlight. The pathogenic microörganisms or viruses which e commonly disseminated by droplets of moisture are those of hooping cough, mumps, measles, influenza, epidemic meningitis, neumonia, and pulmonic plague.

Air-borne infections rarely occur, as previously stated, and are not of eat importance in the open air where sunlight has free access to the sease germs but this type of infection sometimes occurs in crowded arters such as dark shops, schools, tenements and railway trains. owever, the factor of direct contact must be given its due weight in ch instances.

Water-borne Infections.—Pure infections of this type occur in prac-ally only five diseases, namely, Asiatic cholera, typhoid fever,

paratyphoid fever, and in dysentery of the amœbic and bacillary forms The length of time that these microörganisms will remain alive in th water depends on the quantity and quality of organic matter present Only under rare circumstances do bacteria proliferate in water c very high organic matter content. Ordinarily microörganisms wi live only a few days if the water is absolutely pure. They have, how ever, been known to live for several weeks in ordinary river water. Th drinking of water or of fluids or material contaminated by water i the common but not the only way these diseases are acquired.

Infections from Soil.—The soil as a source of infectious micrc örganisms is of importance in only a few diseases, namely, anthra tetanus, symptomatic anthrax, malignant edema, emphysematou gangrene, Asiatic cholera, and typhoid fever. In the first five men tioned infection always takes place through some wound usually i the skin and in the last two diseases mentioned infection is usuall through the intestinal tract but may also occur by means of wounds The microörganisms of anthrax, tetanus and emphysematous gangrene or more specifically the spores, will remain in soil for long periods c time. They are sometimes found in the active vegetative stage bu it is probable that they do not proliferate to any extent in the soi They exist as ordinary saprophytes. The microörganisms of typhoi and cholera have been known to remain alive for a year or more i soils containing large quantities of organic matter. The variou pyogenic micrococci are also occasionally found in the soil and ma enter the body of man and animals through wounds. These last mentioned organisms may live for indefinite periods of time on th skin and enter the body only when the resistance of some tissue i lowered.

Infection from Food.—Quite a large variety of pathogenic micrc örganisms have been found in the various food products. Milk i perhaps the most common food product to be infected. The cause agents of diphtheria, scarlet fever and some other diseases have bee disseminated by means of milk. Milk contaminated by water con taining *B. typhosus* may be the means of conveying typhoid fever, an the dissemination of Malta fever is accomplished by the drinking of th milk of infected goats. Typhoid fever has also been known to hav been acquired from the eating of vegetables which have been washed i water containing the pathogens. Oysters and various shell fish hav

be known to carry the microbic agents of typhoid fever and Asiatic cholera. Three infections coming from meat sometimes occur, namely, botulism, enteritis and occasionally paratyphoid fever. In these instances the causal microörganisms are in the meat. Another type of infection known as ptomain poisoning also occurs from the eating of meat or fish which has been acted upon by saprophytic bacteria and the proteins split up into toxic substances.

Animal Carriers of Infection.—Animals may communicate disease microörganisms to one another and to man in three ways, namely, first, by direct or indirect contact; second, by serving as mechanical carriers from one individual to another; and third, by serving as intermediate hosts for the microbic agent and then subsequently communicating it to another. As examples of the first proposition, the fact that tuberculosis has been communicated from cattle to man, that glanders has been communicated from horses to man, and that anthrax has been communicated from sheep to man by contact may be mentioned. It has also been stated that the cat, while not suffering from true diphtheria, seems to be able to transmit this infection and the dog may also transmit rabies to the man. In the second method of transfer, the mechanical carrying of an infection, the insects are principally concerned. It is well demonstrated that common flies frequently carry *B. typhosus* on their feet from the infected patient or the excreta and deposit them on the food materials thus causing infection when the food is eaten. The various suctorial insects also may suck up the blood of one individual and carry the infectious agent to the normal individual. Notable examples of this are found in the transmission of the various trypanosomiases by the tsetse and other tropical flies, and of Rocky Mountain spotted fever by the wood tick. The same is true of *Bact. pestis* of plague which is carried by the flea, of Texas fever by the cattle tick, and it has been shown recently that the louse may be one of the agents in the transmission of typhus fever. As an example of the third method, the serving as an intermediate host and the carrying of the causal agent, the mosquitoes which serve as the only means of transmission of the causal microörganisms of malaria and yellow fever and in which these parasites pass a certain cycle of their existence, may be mentioned.

Human Carriers of Infection.—It has been mentioned previously that man is capable of carrying infectious agents when he himself is

not infected. For example, in the case of diphtheria it has been repeatedly shown that convalescents from diphtheria, persons who have had the disease, and persons who have never had the disease, frequently carry the etiological microörganisms of this disease in virulent form and are accordingly exceedingly dangerous as disseminators. Not uncommonly persons who have had typhoid fever carry large numbers of virulent *B. typhosus* in their bodies, particularly in the gall-bladder, and disseminate them through the intestinal excreta thus causing many infections when this excreta comes into contact with water used for drinking purposes or food supplies. *B. typhosus* may be carried for many years, one case of twenty years being on record. Asiatic cholera is occasionally carried in the same way. It has also been shown that well individuals may carry the etiological agents of epidemic cerebrospinal meningitis and acute poliomyelitis or infantile paralysis. Individuals who carry infecting organisms are popularly known as "bacilli carriers" and should always be kept under rigid quarantine or observation.

Contact Infection.—It is only necessary to emphasize certain points in addition to what has been said in the foregoing. It has been stated that animals may communicate an infectious agent to other animals of the same or different, but susceptible, species by direct contact. Probably the commonest diseases to be communicated by animals to each other are tuberculosis and glanders. This is commonly accomplished by the rubbing of the mouths and noses together although the disease may be acquired in other ways. Among the human race the diseases which are usually communicated by the contact of one individual with another are diphtheria, scarlet fever, smallpox, chickenpox, mumps, measles, gonorrhea, chancroids and syphilis. In the first mentioned diseases it seems that the expirations and possibly in rare instances the desquamations of the skin in those which have an eruption carry the causal microörganism. The infectious agent is inhaled into the nose or throat. Some of these diseases may be in rare instances transmitted by intermediate agents, clothing, etc. (fomites). In the last three diseases mentioned, which are known as the venereal diseases, an abrasion of the integument is a prerequisite, and the infectious agent must enter by this route. Infection is usually brought about by absolute contact of one individual with another.

leprosy also almost direct contact is necessary for a transfer of the ectious agent.

e Routes by Which Infectious Microörganisms Enter the Body

Microörganisms enter the body through either the external or ernal surfaces. It has been shown that the absolutely healthy and act skin furnishes an efficient barrier to the entrance of infectious nts. Pathogenic bacteria, for example, the streptococcus and the ious varieties of the staphylococci are present on the skin almost tinuously yet do not often produce infections. When there is an asion of the skin or a diseased duct or hair follicle the bacteria quently pass down into the skin and an infection results. When t pyogenic bacteria, such as mentioned above, are vigorously rubbed o the skin infection sometimes takes place but in this instance also re has been some mechanical injury to the skin. Minute unobserved asions of the skin also serve commonly as points of entrance f the *Bact. pestis* of the plague. The microörganisms of tetanus, hrax, symptomatic anthrax and malignant edema always enter t skin through wounds. Sometimes the infectious agents remain l and at other times are carried from the point of the original rance. This may take place in different lengths of time. For mple, in tetanus the bacteria remain localized in most instances at t point of the original wound and their toxin diffuses from this p t. In the various pyogenic infections the bacteria usually remain l lized. However, in anthrax the bacteria are carried into the circu- lon in a very few minutes after they enter the wound. In the new- b n infection very frequently enters the body through the umbilicus o avel. Tetanus is one of the common diseases acquired in this way i ertain localities. Microörganisms may also enter the skin through t wounds made by insects such as mosquitoes, flies, ticks, and fleas. T larger and the clearer cut the wound the less the danger of infec- ti because of the mechanical and bactericidal barrier of the fibrin and t bactericidal action of the blood serum. A free flow of blood also w hes the microörganisms out of the wound. Crushing wounds are e cially dangerous inasmuch as there is not a free flow of blood and a there is a good chance for the growth of anaerobic bacteria such

as those of tetanus, malignant edema, symptomatic anthrax and emphysematous gangrene.

The mucous membranes of the nose, throat and mouth are quite resistant to infection. The epithelial coat, the mechanical action of the mucus and saliva and possibly the slight bactericidal action of the saliva are the barriers. Infections of the thin non-resistant mucous membranes of the new-born do occur and necrosis sometimes results (noma). The mucous membrane of the mouth and throat is frequently the seat of primary infection when it is injured. The actinomyces fungus usually enters a lesion in the mucous membrane made by straws and other substances. The ducts of the salivary glands also serve as points of entrance for certain infectious agents. The tonsils are very commonly the seat of infections especially with the *Strept. pyogenes* and *Strept. pneumoniæ*. Septicemias, as for example those occurring in diphtheria, and especially in scarlet fever, frequently arise from infection of the tonsils with *Strept. pyogenes*. These structures are also the primary point of invasion in cases of acute rheumatism and possibly in certain cases of pulmonary tuberculosis. The nasal mucous membrane is undoubtedly more permeable to infectious agents than that of the oral cavity. The microörganisms of acute epidemic meningitis, acute poliomyelitis, measles, leprosy and glanders undoubtedly most frequently enter the body through lesions in the membranes of the nose. Infection may be carried into the nose directly or pass from the conjunctiva through the naso-lachrymal duct.

The flora found in the eye is quite extensive. The conjunctiva is frequently the seat of primary infections. The pyogenic cocci and the *M. gonorrhoeæ* are among the common infecting agents. It is possible that certain points of infection are provided by the conjunctiva being injured by dust particles. The tears are not bactericidal and only serve to mechanically wash the eye. Infections of the conjunctiva are frequently very severe. There is no doubt also that other pathogens are caught in the eye and washed into the nose where they set up infections or are carried through the membranes to set up infection elsewhere. *M. intracellularis var. meningitidis* of epidemic meningitis is known to pass in this way and possibly *Bact. pestis* of the plague in certain instances.

Infectious microörganisms after being taken into the body through the nose or the mouth may either pass to the lungs through the trachea

down the oesophagus to the stomach and intestines. During the dinary inspiratory part of a respiration it is probable that micro-ganisms cannot pass directly into the alveoli of the lung as the rtuous passage, the mucus and the cilia are fairly efficient barriers. acteria may be inhaled directly into the finer bronchi and the alveoli uring forced inspiration such as that attendant upon hiccoughing and eezing. Infections of this kind occur in pneumonia, tuberculosis, nd influenza. Microörganisms frequently lodge on the membrane the trachea and are here taken up by the leucocytes and carried to ie lungs, bronchial lymph glands, and occasionally to other parts of ie body by the blood and lymph. It is probable that such a form of fection occurs sometimes in pneumonia, tuberculosis and plague.

Infectious microörganisms very frequently pass down to the omach and intestines. The mucous membrane of the stomach normally very resistant to infection due to the hydrochloric acid hich is normally present in the gastric juice and which in normal nount is distinctly antiseptic. It should be remembered that in stances where the acidity of the stomach is lowered that micro-ganisms will develop. All toxins with the exception of that of . *botulinus* of meat poisoning are destroyed by the gastric juice. he intestines are less resistant to infection. It is here that the usal microörganisms of typhoid fever, Asiatic cholera, chicken olera and dysentery and the various hemorrhagic septicemias find eir particular affinities. These bacteria enter or attach themselves the intestinal wall and in the case of cholera and dysentery this the only point of infection. The *B. typhosus* has occasionally en known to enter at other places. This bacterium, however, com-only localizes in the lymphatic patches (Peyers) of the intestine, d may in certain instances enter the blood from this point. It ould be noted that some bacteria can pass through the wall of the testine when it is seemingly intact. This point has been repeatedly monstrated in the case of *Bact. tuberculosis.*

The genital organs of the male and female are susceptible to in-ction with microörganisms in certain instances. The *M. gonorrhœæ* gonorrhœa and the *Treponema pallidum* of syphilis find their usual rtals of entry in the genital tract. They have, however, been known infect other parts of the body as the mouth, rectum, and the con-nctiva. The etiological bacteria of gonorrhœa can penetrate the

seemingly intact male urethra but not the vagina of the female c account of the bactericidal secretion and the character of its squamo epithelium. Other bacteria, as for example, the *Strept. pyogenes*, *S pyogenes var. aureus* and *B. coli* are sometimes found infecting t genital tract in cases of chronic urethritis.

The kidneys, ureters and bladder are sometimes infected but usual the infecting agent is carried in the circulation although it occasional ascends through the urethra from without.

In conclusion, the proposition of germinal and antenatal infecti must be mentioned. By true germinal infection is meant the carryi of the infectious organisms of a disease by the ovum or the spermatoz and its incorporation in the development of the embryo and fet It is doubtful if this ever occurs. Some authorities claim that it possible and that it has been demonstrated that the spermatozoa m carry the *Treponema pallidum* of syphilis, but this is not general accepted. Antenatal infection or infection of the fetus before bir does occur. Infectious organisms enter the fetus only as a result intimate contact with the mother and it has been repeatedly sho that tuberculosis and syphilis may be acquired in this way. It essential, however, that the mother be infected and in most instanc this infection is localized in the placenta. Smallpox, scarlet fev measles, dysentery, various pyogenic infections, and in rare instanc pneumonia, have also been acquired by placental infection. In ra cases in animals, anthrax, symptomatic anthrax, chicken cholera, a glanders have been acquired by antenatal infection from the moth

Variation in Infection

It should be noted that there may be a variation in the infecti depending upon the route by which the infectious microörganism ente the body. For example, in the case of *Bact. tuberculosis*, if the bacteriu enters the skin a usually non-fatal infection called lupus vulga results; if it enters the lymph glands or joints and localizes there a inflammation of not necessarily a fatal character results; if it ente the lungs pulmonary tuberculosis or consumption occurs which us ally, after being well established, runs a fatal course; if it enters t intestine, intestinal tuberculosis may result which is nearly alwa fatal; and if it enters the meninges, tubercular meningitis results whi

rapidly fatal. Just so with the *Strept. pyogenes*, depending on hether it enters the circulation, the lymphatic vessels of the skin or ıe connective tissues there results septicemia, erysipelas, or abscesses hich obviously differ in their severity. The same is true of practically l the pathogenic bacteria which invade the plant and animal body, ıe variation in the route produces a great variation in the type and ıe results of the infection.

It was mentioned in the beginning of this discussion that *infection* cluded certain things such as the entrance of bacteria into the body ssues, their increase and their injury to the body. There is some ariation in what constitutes an infection depending upon the infectious icroörganism and the tissue it attacks. For example, *Msp. comma* Asiatic cholera does not produce an infection unless it comes into ntact with the intestinal mucosa and in this case it does not enter ıe tissues but sets up an inflammatory process on the surface. If ıis same bacterium comes into contact with tissues such as those of ıe nose, throat, lungs, no infection results. In the case of *B. typhosus* typhoid fever the bacterium not only attacks the intestinal mucosa, ıt in addition it enters the tissue of the lymphatic patches and sets p an inflammation. This microörganism may also invade the rculatory system directly. In order for such an organism as the *trept. pneumoniæ* to produce pneumonia it is only necessary for the acteria to come into contact with the thin, single-celled, alveolar wall rough the blood or air passages. In case this bacterium produces an oscess it is necessary for it to first enter into the tissues. In the neumonic form of plague, although infection is supposed to be ac-mplished generally by the inhalation of bacilli, the *Bact. pestis* may e carried to the alveolus through the circulation and thus enters the ssues of the lung before actually invading the alveoli. This some-mes occurs in case of *Strept. pneumoniæ*. It also gives rise to abscesses ccasionally but only when it invades lymphatic glands. The same is ue of the large number of infectious microbic agents; there is a varia-on in the infection due to the variation in the microörganism and ıe point where this agent attacks the body. The severity of an ıfection, as for example, a pneumonia due to *Strept. pneumoniæ* or *Strept. pyogenes*, or to *Bact. pneumoniæ* (Friedlander) or to *Bact. estis*, would vary with the infectious agent, its virulence and number, ıd with the resistance of the individual infected.

The Factors which Influence the Results of an Infection

There are four principal factors which influence the results of a infection. They are as follows: The virulence of the infecting micr örganism; the number of the infecting microörganisms; the avenue b which the infectious microörganism enters the body; and the resistanc of the plant, animal or individual infected.

Virulence.—It is a self-evident fact that the more virulent a micr örganism is the more serious will be the infection which results from it invasion of the body. There is a great difference in the virulence. Fc example, *Strept. pyogenes* may infect the skin of mucous membrane and produce only an abscess of varying proportions. Again, it may b more virulent. The resistance of the infected individual may b lowered somewhat and the streptococcus may enter the lymphatic of the skin and produce erysipelas or the blood stream and produc a fatal septicemia. Furthermore, one strain of the streptococci i the blood may produce a very virulent infection and another a les severe one. Virulent streptococci are not phagocytized by the leu cocytes. The same variation in virulence is noted in all the pathogeni bacteria and the infections are modified thereby. The fact that a organism is virulent for an animal is not evidence that it is virulent fc man. The virulence of an organism depends upon its ability to forr toxins or other poisonous substances.

Number.—The number of infecting microörganisms which ar introduced into an animal body is of importance. In anthrax, fc example, it has been shown that one bacterium is capable of multiply ing and setting up an infection. In tuberculosis and typhoid fever an most of the infectious diseases it requires a rather large number befor an infection will take place. The leucocytes, bactericidal substance in the blood, and the body cells in general are capable of destroying man infectious agents. Furthermore, it can be readily understood how few bacteria might be able to cause a mild infection and an increasin number be able to so overcome the bodily resistance as to cause a mor or less severe infection.

Avenue.—It has been pointed out previously how the avenue c infection modifies the infection. A very virulent microörganism ma occasionally produce a very mild infection when introduced in certain locality while in another place the same organism may produc

very severe type. The results of the infection will be materially modified depending on the avenue of entrance which the virulent microrganism takes. For example, in addition to those mentioned previously, suppose *Bact. pestis*, the causal agent of plague, enters the blood through the skin, or the lymphatics through the tonsils, it is carried to the lungs and there produces a very severe and usually fatal pneumonia; if bacteria enter the lymphatic system in large numbers they frequently localize in the lymph glands producing buboes or glandular enlargements which are not always fatal. These bacteria may also enter the blood current and produce a rapidly fatal septicemia. It has not been established in man that plague can be produced by the ingestion of *Bact. pestis*, but in some susceptible animals such as rats, the disease in a very fatal form is rapidly acquired when the bacteria enter the intestines.

Resistance.—This factor is one of the prominent ones which modify the results of an infection. It is a familiar fact that two or more individuals may be infected with the same microörganism, as for example, *B. typhosus* and one will not become infected or have a very mild form of the disease, while the other will have the severest and most fatal form of typhoid fever known. Again, the age of the individual infected is important in determining the resistance. The adult resists infection such as diphtheria, scarlet fever, and measles more than the child. The very young child resists pneumonia and tuberculosis more than the adult. The resistance of the body depends on the presence in that body of natural or acquired antibodies. It is, therefore, obvious that the higher resistance or immunity of the individual infected, the less severe will be the results of the infection on that individual.

The Exact Cause of Infections

We are familiar with the fact that all of our infectious diseases are due to microörganisms or viruses of some form or other. The causal agents of many of these diseases are known but in the case of those that are not known there is reasonable certainty as to the types of the infecting agents. The exact substances which are produced by the microorganisms and which are responsible for symptoms of the various diseases will be briefly considered in the following paragraphs.

SOLUBLE TOXINS.—It is a known fact that there are some pathogenic bacteria which secrete through their cell walls poisons which diffuse into the surrounding media. To these poisons or toxins the disease symptoms are due. *Bact. diphtheriæ* of diphtheria, *B. tetani* of lock-jaw or tetanus, *Bact. dysenteriæ* of bacillary dysentery, *B. botulinus* of meat poisoning, and *Ps. pyocyanea*, the causal organism of blue-green pus, are about the only bacteria of this character. Some bacteria, such as *Strept. pyogenes* and *M. pyogenes var. aureus* produce hemolytic toxins. There are certain protozoa as, for example, certain entamœbæ and the various trypanosomes which secrete soluble poisons. Among the animals, the venoms of the poisonous snakes, the poison of the centipedes and spiders, the serum of the eel, and the excretion of the dermal glands of the toad are examples of secreted toxins (zootoxins). Again, among the plants are abrin from the jequerity bean, ricin from the castor oil bean, and others, are examples of soluble toxins the product of plant cells (phytotoxins). The cells producing these toxic substances, therefore, are only indirectly responsible for the infections for it is the toxins themselves which produce the pathogenic effect on the body.

ENDOTOXINS.—Many of the pathogenic bacteria and some of the protozoa do not secrete their toxins outside the cell wall but hold them within the wall in combination with the protoplasm. They do not liberate these substances until the microörganisms die and are disintegrated. Such toxic substances are called *endotoxins* to distinguish them from those secreted from the cell, namely, the *soluble toxins*. Two of the best examples of pathogenic bacteria of this type are the *Msp. comma* of Asiatic cholera and *B. typhosus* of typhoid fever.

TOXIC BACTERIAL PROTEINS.—There are some bacteria and other parasitic cells which produce a small amount of endotoxin and in certain instances some soluble toxin but not enough of either of these substances to account for the toxicity of the organism. It has been found that when organisms of this character are ground up and washed to free them of their endotoxin and are washed free of all soluble toxins, they are still toxic. It has been shown that this toxicity is due to the protein substances of the cell. The *Bact. tuberculosis* and the *Bact. mallei* of glanders are two notable examples of microörganisms of this character. When, for example, the proteins of *Bact. tuberculos*

are injected into the circulation of susceptible animals, tubercle formation occurs showing that these proteins are poisonous.

Other Possible Exact Causes.—In certain infectious diseases it is also claimed by certain writers that enzymes are responsible. This lacks substantiation. It is also stated that in such infections as anthrax that the mechanical effect of the bacteria plugging up the capillaries and producing mycotic emboli is a factor. This may be true but in addition other factors are concerned as previously mentioned.

In mixed infections of two or more organisms, which frequently occurs, the infected individual may have within the body soluble toxins, endotoxins, and toxic bacterial proteins and in such a case it is difficult to differentiate their action.

The Methods by which Infectious Microörganisms are Disseminated

The microörganisms of some of the infectious diseases such as diphtheria and Asiatic cholera and usually tetanus remain local and seldom enter the body generally. From the locus of the infection they disseminate their toxic or poisonous products. In the case of tetanus the toxin is carried over the body along the sheaths of the motor nerves; in diphtheria the toxin is usually carried by the lymph, occasionally by the blood; and in the case of cholera the blood and lymph both serve to carry the toxic agents. In diphtheria and cholera the microörganisms very frequently extend along the mucous membranes from the original point of infection. There are other infections in which the causal microörganisms extend only from the point of original invasion into the surrounding areas. Such is the case with *Strept. pyogenes* in the infection of the lymphatics of the skin in erysipelas and of *Bact. influenzæ* in all infection through the respiratory tract. Many of infectious agents are carried by the blood and occasionally by the lymph, as for example, in tuberculosis, syphilis, glanders, plague, leprosy, pneumonia, and the septicemias due to the pyogenic cocci. It is possible in certain cases that the leucocytes acting as phagocytes may carry virulent infectious agents through the blood and lymph from one part of the body to the other.

The Methods by which Infectious Agents are Eliminated from the Body

The etiological microörganisms of the various infectious disease may be eliminated from the body in two general ways, namely, by direct method and by an indirect method. For a microörganism t be directly eliminated from the body it is necessary for the focus of th infection to communicate with the outside of the body in some way o other. In the case of infections of the mucous membranes and the ski there is, of course, direct communication with the outside. In disease of the respiratory organs and the intestines the infectious agents ar discharged into the lumen of the air passages and the intestines and the thrown out from these passages. Examples of the partial direct elimi nation from the skin may be found in such diseases as smallpox, measles syphilis, scarlet fever, lupus vulgaris, and in suppurative condition such as carbuncles and furuncles. From the present evidence littl significance perhaps is to be attached to the elimination of the infectiou agents mentioned directly from the skin. It is probable that the micro örganisms which are eliminated remain alive for only a short time and ar not factors of consequence in the transmission of these infections. A examples of diseases in which direct elimination from the variou mucous membranes occurs infections such as typhoid fever, tubercu losis, cholera and dysentery from the membranes of the intestines influenza, pneumonia and tuberculosis from the bronchial membranes diphtheria, leprosy, glanders and scarlet fever from the membranes o the nose, throat, and tonsils; and gonorrhea, syphilis and tuberculosi from the membranes of the genito-urinary tract may be mentioned In elimination from the various internal membranes sometimes re infections occur such as in the case of the elimination of *Bact. tubercu losis* from the respiratory tract, the swallowing of the sputum, and the subsequent infection of the intestines.

In the second, or indirect method of elimination, two distinct prop positions present themselves; first, the infectious microörganism must enter the lymphatic or blood circulation; and, secondly, in order to get out of the body they must pass through the cells of some of the organs the mucous membranes or skin. It is a common occurrence for bac teria and other microörganisms to get into the circulation in some of the infectious diseases such as typhoid fever, pneumonia, plague,

nd in the various septicemias. They may pass through the epithelium f the kidney and be eliminated in the urine; they may pass through the ver and be eliminated in the bile, finally passing out through the in- estines; and they may pass through the mucous membranes of the ntestine and possibly pass through the glandular epithelium of the ebaceous and sweat glands and be eliminated through the skin. They ave also been known to pass through the glandular epithelium of the ilk glands when these glands are not grossly diseased and through the alivary glands. It has been recently well demonstrated that there ust be some form of lesion in the liver and kidney in order for the icroörganisms to pass through. Infectious microörganisms are some- mes destroyed by the lysins in the blood, carried to and deposited the spleen and bone marrow and gradually disintegrated and issolved.

In certain infections in which a recovery seems to have occurred ll the infectious microörganisms are not always eliminated from the ody. As mentioned previously, *B. typhosus* and *Bact. diphtheriæ* are equently carried by persons fully recovered from these diseases. ometimes, however, inflammatory infections are set up by these bac- eria. It has been suggested on seemingly good evidence that inflam- nations of the gall-bladder and gall-stone formation may be due to the oxic action of the bacteria of typhoid fever which have been retained the gall-bladder for a considerable time following an attack of typhoid ever. It is known that frequently repeated attacks of malaria are due o the retention of some of these protozoan parasites for a time in the uiescent stage. Repeated attacks of erysipelas caused by the *Strept. yogenes* may also be due to the same condition. It is also claimed by ome (Von Behring) that *Bact. tuberculosis* is taken into the body in nfancy, that it is not eliminated, and that it sets up infection in later fe.

In conclusion should be mentioned one other indirect way in which nfectious agents are eliminated from the body, namely, by being aken up by suctorial insects from the blood. It is necessary that this e done in order to perpetuate the parasite and complete its life cycle in ertain instances, as with the mosquitoes in yellow fever and malaria. n other instances the parasites are only taken up by the insect and ubsequently injected into another individual or digested as the case ay be. This occurs with the ticks in the transmission of certain of

the piroplasmoses and with the tsetse flies in the transmission c certain of the trypanosomiases.

The Effect of Infectious Microörganisms on the Body

It becomes necessary to consider briefly the effect of the variou infectious microörganisms and their toxic substances on the body.

The Period of Incubation.—This period is that elapsing afte the entrance of the infecting organism into the body until the symp toms of the disease develop. This period is variable in most disease and depends upon the same factors which modify the results of a infection, namely, the virulence of the infecting organism, the numbe growing in the body or its tissues, the avenue, and the resistance of th individual. The period can be in a measure controlled and shortene in experimental animals by inoculations into the circulatory syster and in other regions depending on the organisms used. In some c the human diseases, particularly those of unknown cause, the period c incubation is quite constant, as for example, in smallpox and measle

Local Reactions.—The local effects of the toxic substances c microörganisms are usually first inflammatory and later possibly ne crotic, that is, they produce a death of the tissue involved. The inflam matory changes may be confined to those of an acute character as, fo example, in the various serous, hemorrhagic, suppurative and fibrinou inflammations, or be chronic and proliferative in nature. There i always a variation in the type of inflammation depending on the loca tion of the infection and also a variation in two different individuals c the same species infected at the same point with the same agent. I some diseases such as tetanus the local point of infection may entirel heal and still the bacteria be localized at this point and disseminat their toxin. In some cases of tuberculosis and glanders the bacteri may become localized at the points of infection and after an acut inflammatory stage the point may become the seat of a chronic proces and proliferative changes occur in the tissues.

General Reactions.—*Metabolism.*—The general metabolism o the body is affected by the changes produced in the amount and th chemical constitution of the food substances which are taken int the body. By changing in the same way the substances which natu rally are eliminated from the body and by setting up new and abnorma

anges in the functional activity of the tissues, the general metabolism ay be disturbed. Muscular weakness, delirium, pain and loss of petite, together with vomiting, diarrhea, disturbance of intestinal sorption and the digestive juices are often noted in cases of altered tabolism. The fats, carbohydrates, and then the proteins are in e order named rapidly used up, producing certain changes in the pired air and in the urine and feces. Infectious microörganisms ay also reduce the power of the hemoglobin to carry oxygen and haps cause a narrowing of the respiratory passages thus preventing e necessary amount of oxygen reaching the lungs and subsequently e tissues.

Infecting microörganisms may alter the composition of the food bstances and after being taken into the body produce abnormal mpounds which have little or no nutritive value on absorption or ey may produce toxic substances related to ptomains. Substances hich normally should be eliminated from the body are often retained d abnormal losses of such substances, as water and various albumi-us compounds, occur.

Attendant upon the changes in metabolism usually there occurs *ver* in all infectious diseases. It is probable that the fever is the sult of the effect of the toxic protein compounds of the infecting icroörganisms on the tissues, or the disintegration of the protein mpounds of the body due to the action of toxins. It is evident that e fever-producing substances, in certain infectious diseases, act in a ry characteristic manner as is demonstrated by the so-called typical ver curves. It seems to have been demonstrated that fever is a good gn and that it is indicative of the reaction of the body to the toxic bstances of the infecting agents. It has also been shown that the ll of fever in certain infections is attendant upon the formation and turation of the body fluids with antibodies.

Blood-forming Organs.—There are usually changes in the blood-rming organs in most all types of infection. The spleen frequently ows enlargement and contains numbers of myelocytes which are so found in the blood in increased numbers. This is probably due to e disintegration and deposition there of red blood corpuscles and so to the action of toxic substances. The endothelial cells and locytes of the spleen are actively phagocytic. The bone marrow, rticularly the fatty marrow, shows large numbers of myelocytes of

the neutrophile type and it eventually becomes lymphoid in natu in a large number of infections. The lymph glands also frequent show endothelial proliferation.

Parenchymatous Tissues.—All sorts of degenerations of the kidney heart, liver and some of the other organs frequently occur in infectio Amyloid formation and necrosis of tissue sometimes occurs. Many the toxins have special affinities for tissues, such as the tetanus tox for nerve tissue and this produces organic changes. It is possibl also that the fever is responsible for a certain portion of the chang in the parenchymatous organs in infections.

Epithelial and Endothelial Tissues.—In certain infections, as f example, diphtheria, the epithelial tissues are subject to inflammatio and in other infections, as for example, syphilis, the endothelial tissu of the blood-vessels undergo inflammation and sometimes proliferatic The epithelial and endothelial cells are frequently actively phagocy and large numbers of the infecting microörganisms are taken up a destroyed. Some of the infectious microörganisms produce no effe whatever on these tissues, while others produce pronounced destructi changes.

Erythrocytes and Leucocytes.—Lytic or dissolving substances f the red blood corpuscles are frequently produced in infections (hem lysins). *Strept. pyogenes*, *M. pyogenes var. aureus*, and *Ps. pyocyan* are among the bacteria which produce hemolysins. Anemia is, therefo not an uncommon finding in many infections. Normal human blo and that of some animals contains an antilysin for the staphylolysin a it is sometimes produced in large amounts. Agglutinating su stances for red corpuscles are produced by some pathogenic micr örganisms and it is possible that these are the cause of the so-call agglutination thrombi which occur in infections like typhoid fever.

The most marked changes seen in the leucocytes in infections their rather constant increase in number; in most cases a leucocytos In uncomplicated tuberculosis and typhoid fever, in measles a German measles, in malaria, and in dengue, there is no increase number. In acute inflammations it is the polymorphonuclear leuc cytes that undergo an increase. This increase is sometimes preced by a decrease (leucopenia). The leucocytes act as the principal phag cytes of the body and are attracted (positive chemotaxis) to the bacter or other microörganisms after they have been sensitized by the opsoni

the body fluids. Besides acting as phagocytes they may, according to Metchnikoff, produce antitoxins and bactericidal substances. It has been suggested that the initial leucopenia in some cases is due to the production of negative chemotactic substances. Some virulent bacteria cannot be phagocytized probably because they produce very strong negative chemotactic substances.

Antibody Formation.—One of the general effects of infectious microorganisms in certain infections is the production of antibodies of various kinds. These may be antitoxins as in the case of tetanus and diphtheria, or bactericidal substances as in typhoid fever and cholera, or tonic substances as in the pyogenic infections. Agglutinins, precipitins, and other bodies are also sometimes produced in conjunction with the other immune substances mentioned.

CHAPTER II*

IMMUNITY AND SUSCEPTIBILITY

General

Definition.—A clear understanding as to what is meant by t terms immunity and susceptibility is of fundamental importan By *immunity* we mean the resistance which an animal or plant bo possesses to the etiological microörganisms of an infectious disease a to the disease itself. The name has been adapted from the La *immunis* which meant a person who was free or exempt from pub duties and later, one who was exempt from the action of poiso Briefly stated, *immunity is resistance to disease.* It results commonly a natural termination of the process of self-healing in many infectic diseases. The absence of such resistance, which may be total or parti characterizes what is known as *susceptibility.* Throughout the anin kingdom and also among the plants there is a great variation in t immunity and susceptibility in the different species to the various d eases. Immunity bears no relation to the contagiousness of a dise and the term is only applied as a rule to strictly infectious diseases a not to metabolic diseases.

Hypersusceptibility or Anaphylaxis.—It has been shown th animals and man are occasionally hypersusceptible to certain protei For example, there are individuals who are always seriously poison by the ingestion of eggs, pineapples, and strawberries. Certain in viduals when injected with diphtheria or tetanus antitoxin which carried in horse serum are seriously intoxicated and occasionally d In such instances the proteins of the horse serum and not the antito are responsible. It has been demonstrated that an animal may sensitized or rendered hypersusceptible to almost any protein by fi injecting a minute amount of the protein and then, after a period of least eight to thirteen days, may be seriously intoxicated, if not kill by the injection of a slightly increasing dose of the same protein. T

* Prepared by E. F. McCampbell.

proteins of the bacterial cells have been shown to act in the same way. Animals injected, as described above, may be rendered hypersusceptible to all bacterial proteins. Furthermore, as referred to above, individuals may be naturally hypersusceptible to bacterial and other proteins. The manner of the original sensitization in these cases is not known. Sensitization which has been either naturally or artificially acquired is transferred in most instances *in utero* to the first generation; that is, a mother may be sensitized, convey the sensitizing substances to her young while in the uterus, and when these offsprings are subsequently injected after birth with the same protein they may be intoxicated or killed. In this connection it should be stated that the so-called inherited tendency to specific diseases may be something more definite than we are ordinarily accustomed to regard it. Suppose a mother becomes tuberculous and is, therefore, sensitized to the proteins of *Bact. tuberculosis;* it is quite possible for her to convey to the offspring this susceptibility to the particular proteins of *Bact. tuberculosis* as has been demonstrated artificially. After birth, or in later life when the causal microörganisms of tuberculosis are taken into the body, as they are in about ninety-five per cent of all persons in civilized countries, the bacteria may find a more than ordinarily susceptible individual and may develop with comparatively little hinderance. If this condition be true naturally as it is when produced experimentally in susceptible animals a very interesting and scientific explanation of the so-called inherited tendency to tuberculosis is at hand.

Predisposition and Non-inheritance of Infectious Diseases. —There is probably no such thing as a truly inherited infectious disease. This point has been debated and discussed for a great many years and the above conclusion has been reached by the majority of investigators. By *inheritance* is meant the transference of a property, or in this instance, a pathogenic microörganism by the nuclear substance of either the spermatozoön or the ovum. It is only the nuclear substances which combine to form the new individual. It is true among certain of the lower animals, such as the fowls and some insects, that microörganisms are carried within the egg but the eggs are quite different in structure from the human or mammalian ovum. The egg of the above-mentioned animals is composed largely of yolk-furnishing food and there is ample opportunity for microbic growth, while the mammalian ovum contains no yolk. Such instances should be referred to as *germ-cell transmission*,

not inheritance. If the microörganisms were present they would immediately incorporated within the new developing embryo. If t microörganism ever did find its way into the human or mammali germ cells it would be a mechanical impossibility for the cells of t embryo to divide and multiply in proper manner. Such pathoge would rapidly destroy the developing cells in the embryo. It is tr that the offspring of certain individuals are born diseased. For e ample, children are not infrequently born with syphilis and tuberculos At first thought it might seem that this is inheritance but on care analysis it will be found that the mother is either syphilitic or tuberc lous. Furthermore, the locus of the infection is most frequently in t uterus and the microörganisms are transferred to the unborn offspri by means of the fetal circulation. This condition is what is known *antenatal acquirement;* it is not heredity. It is absolutely impossible f the male to communicate any disease to the offspring unless the fem is first infected. Colle years ago formulated a law which bears his na in which he stated that a father could communicate syphilis to his ch without the mother being infected. This law has been disproved sin the introduction of the new serum tests for syphilis and it can be po tively demonstrated in all such cases that the mother is infected. An or prenatal acquirement may then be recognized. What can be sa in regard to the predisposition to a definite infectious disease? The is a question as to whether true predisposition does exist. Ma cases are on record to show that disease seems to run in families and localities. For example, tuberculosis and cancer are frequently sa to be subject to inheritance or to predisposition in certain cases. can be easily seen that if one parent is diseased the germ cell of t parent will be less healthy and when combined with a normal healt germ cell of the other parent will not give rise to as healthy an individu as when both cells are from healthy individuals. Again, the result wh the germ cells of both parents are unhealthy due to the parents bei unhealthy, is evident. Predisposition seems to resolve itself into t inheritance of a weakened constitution, a constitution which will n withstand the ordinary infections easily. It seems not to be a pred position to any particular disease but a predisposition to all diseas infectious and metabolic. Diseases such as tuberculosis are prevalent that it is very possible that infection may take place and it interpreted as inherited because the parent died of the same cau

mentioned above, it may be that the true explanation of the enomena of predisposition is found in anaphylaxis or the sensitiza-n to various proteins of microörganisms. Further work is necessary ng these lines.

Immunity

Immunity and susceptibility to disease are always relative and ver absolute; that is, it is always possible to produce some sort of an ection in a supposedly immune animal by modifying the conditions der which the animal is accustomed to live. For example, the cken is immune to tetanus but by keeping this animal for some time a temperature higher than its normal it may be infected. The cow not ordinarily be infected with typhoid but when large numbers of *B. typhosus* are injected under the skin an abscess may be produced. ese and many other examples might be mentioned. Our standard mmunity in a particular animal is based upon the conditions as they st naturally and on the average resistance of animals of the same cies.

Immunity to disease may be of two kinds, natural and acquired. *tural immunity* is that resistance which is possessed normally by an ividual. *Acquired immunity* is that resistance which is acquired having an infection, or by being vaccinated, or immunized against infection with the specific etiological microörganism or its antiserum.

Natural Immunity and Susceptibility.—Attention should be cted to certain forms of natural immunity and susceptibility.

Racial Immunity and Susceptibility.—It is a familiar fact that certain cies of animals and certain races of man differ in their resistance and ir susceptibility to infectious diseases. As examples of racial im-nity among animals the native cattle of Austria-Hungary and of an which are relatively immune to bovine tuberculosis, a disease ch causes great loss among other races, may be mentioned. Again, sheep of Algeria are relatively immune to anthrax while all other ep are extremely susceptible. Field mice are immune to glanders le the common house mouse is susceptible. The negro is more re-ant to the infectious microbic agent of yellow fever than other races, is without doubt more susceptible to tuberculosis. The Japanese said to be more resistant to scarlet fever than other races. The

Melanesians are very susceptible to measles and the Malaysians to be beri, while other races are relatively immune.

Familial Immunity and Susceptibility.—It is true that certa families vary in their immunity and susceptibility when compared wi other families in the same community. For example, tuberculo undoubtedly shows a tendency to run in families. In determining case of this kind it is, of course, necessary to take cognizance of t environment of the individual and the association with other diseas persons. The so-called tuberculous diathesis does exist and perhaps have an explanation of it in anaphylactic phenomena as mention previously. Measles and scarlet fever also in certain instances seem run in families.

Individual Immunity and Susceptibility.—Variation among in viduals associated together is noted in regard to their resistance a susceptibility to disease. It is well known, for example, that in a he of cattle, which are in the main tuberculous, there are certain individu who never contract the disease. These animals may be of the sa breed and be fed and handled the same as the rest of the herd, still th never become infected. Again, in the human race, with the acute exa thematous diseases such as scarlet fever and measles, there are childr for example, in the same family and of nearly the same age and livi under exactly the same conditions, who contract the disease and oth who do not. The exact cause of the individual, familial and rac immunity cannot be satisfactorily explained at the present time. Th is also a variation in the individual's resistance at different tim dependent upon food, sleep, work and general hygienic conditions.

Factors of Natural Immunity.—The natural immunity of a individual to an infection may be dependent upon several things s follows:

The Protection Afforded the Body by the Surfaces.—The body surfa may be conveniently divided into those which are external and th which are internal.

Skin and Cutaneous Orifices.—The first protective mechanism th we wish to call attention to is the skin. It is a well-known fact th virulent bacteria are frequently present on the skin of seemingly norm and healthy individuals. Perhaps the most common of these is *Strept. pyogenes* and the *M. pyogenes vars. aureus* and *albus.* The microörganisms and others live largely as saprophytes, feeding up

t dead and desquamating epithelial cells. The skin is impermeable t these microörganisms when it is unbroken in its normal state. Experiments have been performed to determine whether the skin is normally permeable to bacteria. Bacteria have been rubbed into the skin and have produced infection but in these instances the skin has been abraded by the mechanical irritation. Bacteria may infect the sudoriferous and sebaceous glands and their ducts, in case the metabolic activity of these structures is disturbed. The ducts and the glands of the skin are protected ordinarily by a flow of the secretions. In case the flow of the secretions is decreased and the orifices of the ducts contracted as in cold weather, while bacteria find it more difficult to pass down than before, they occasionally do produce an infection. When a hair follicle is diseased and the shaft contracted or perhaps dropped out, bacteria may pass down and produce an infection. *B. teni* of tetanus or lockjaw frequently passes through the skin by means of deep penetrating wounds. The same is true of some other pathogens.

In case bacteria are successful in permeating the skin either directly or by means of cutaneous orifices, they are usually able to set up a marked inflammation of these structures and produce necrosis of the epithelium. It is in this way that pustules, boils, carbuncles, and various forms of cellulitis are produced. The secretions of the sebaceous glands are not germicidal but are perhaps slightly antiseptic due to the salts which are contained therein. Furthermore, as soon as the serum from the blood is extravasated there may be slight germicidal action on the bacteria infecting the skin. The soluble toxins of bacteria cannot be absorbed through the unbroken skin.

The Subcutaneous Tissue.—In case the bacteria are successful in permeating the skin and penetrating the subcutaneous connective tissue, again various protective mechanisms show themselves. This resistance is due to a very rapid production of new connective tissue which serves to mechanically limit the infection. It is due, furthermore, to the germicidal action of the serum, the mechanical and germicidal action of the fibrin and the phagocytic activity of the leucocytes. These various factors will be discussed subsequently in connection with the phenomena and the protective mechanisms of inflammation.

The Exposed Mucous Membranes of the Body.—The exposed mucous membranes of the body usually are covered with a variety of bacteria,

some of which are pathogenic. Their moist condition favors the growth of microörganisms, but the mucus which is secreted upon them forms a mechanical bearer to the bacteria and serves to wash them away. This mucus is not germicidal but is perhaps slightly antiseptic. The only mucous membranes of the body that are really exposed are those of the eyelids, lips, anterior nares, genito-urinary apparatus and the anus. It is perhaps more convenient to discuss these membranes in detail in connection with the cavities which are connected with them.

Nasal Cavity.—Microörganisms find a barrier to the entrance into the nasal cavity in the hairs which protect the anterior nares and serve to keep out the dust from the inhaled air. The membranes of the nasal tract, besides being covered with mucus, which acts as above mentioned, are also covered with ciliated epithelial cells which move from within out and serves to wash the mucus containing the bacteria from the surface. Infections of the nasal mucous membranes are, however, not uncommon. *Bact. influenzæ*, *Strept. pyogenes*, *Staph. pyogenes vars. aureus et albus*, *Bact. diphtheriæ*, *M. intracellularis v. meningitidis*, and occasionally *Bact. mallei* produce infection through this membrane.

The Mouth.—The mouth probably contains the largest variety of bacteria to be found anywhere in the body. A large number of these bacteria are non-pathogenic, although pathogenic microörganisms occasionally occur. All the requisite conditions for bacterial growth are provided in the mouth, namely, temperature, moisture and food. The food supply is largely derived from materials which have been deposited during the process of mastication between the teeth and in the various depressions of the mucous membrane. The microörganisms also feed upon the desquamated squamous epithelial cells. They are being constantly washed off the membrane by the saliva which contains a certain portion of mucus. The saliva is not germicidal, and in all probability only very slightly antiseptic. The most permeable part of the mouth is in all probability the tonsils which separates this cavity from the pahrynx or throat. These lymphatic structures have many deep crypts, and bacteria once entering the tissues of the tonsils may gain access to the lymphatic circulation through these structures.

In case bacteria are successful in passing the obstacles of protection afforded in the nose and in the mouth and pass into the throat, there are two routes for their entrance into the internal body, namely, through

he trachea and bronchi into the lungs and through the œsophagus into he stomach and intestines.

The Lungs.—In case infectious microörganisms pass down the rachea and bronchi they meet first with the obstruction of the mucus vhich is secreted upon the surfaces of these tubes. In addition, ciliated pithelial cells are present and serve to cleanse the surfaces from nicroörganisms as in the nose. Occasionally microörganisms lodge long the trachea and the bronchi and produce slight irritations which if eft undisturbed may immediately produce serious infections. How-ver, the leucocytes from the neighboring bronchial and mediastinal ymph glands pass through the walls of the trachea and bronchi, ingest he microörganisms, carry them back to the glands and in a majority f instances destroy them. Occasionally, however, leucocytes contain-ng virulent microörganisms get into the lymphatic circulation and these re carried by the diffusion currents in the lymph vessels down to the lveoli of the lungs and here may cause inflammations of a more or less erious character. It is probable that the *Strept. pneumoniæ* is very requently carried to the alveoli of the lungs in this way. Without loubt, microörganisms cannot be directly inhaled through the air assages into the alveoli of the lungs during an ordinary inspiration, ut it has been shown that in forced inspirations, such as those attend-ng upon coughing, hiccoughing, sneezing and sighing that they may be o carried.

The Stomach.—In case the microörganisms pass down the œsophagus nto the stomach, they immediately come into contact in the normal rgan, with the gastric juice, which contains the hydrochloric acid in uch concentration that it is at least antiseptic if not germicidal. n case the functional activity of the stomach is disturbed and the ydrochloric acid is diminished in amount, microörganisms may grow n the stomach to a limited extent. Furthermore, in case all the articles of food are not thoroughly broken up in the stomach, bacteria vhich may be contained within these particles may pass through the tomach into the intestine.

The Intestines.—In the intestines the microörganisms come into ontact with the alkaline pancreatic juice which is slightly antiseptic nd with the bile which is antiseptic and in certain instances bacteri-idal. They find no particularly favorable conditions for growth in the pper part of the small intestines under normal conditions. Here

also mucus covers the surfaces. However, if the functional activity of the small intestines is disturbed, bacteria may enter the lymphatic structures (Peyer's patches, solitary follicles) low down in the small intestines and produce infection. Such is the case with *B. typhosus* of typhoid fever and with the *Msp. comma* of Asiatic cholera. Bacteria which have been prevented from development in the small intestines frequently find the opportunity in the large intestine. Here the concentration of the various digestive juices is lowered and the requisite condition for maximum bacterial growth is provided. Nevertheless, infections of the large intestine with bacteria are not common but may occur, colitis of various forms resulting. The various dysentery amœbæ very frequently develop in the large intestine.

The Genito-urinary Tract.—The mucous membranes of the genito-urinary tract, varying in male and female, present the same features as those of other mucous membranes. Besides the secretion of mucus, various other acid-containing secretions are often present. In addition, in the urinary tract the mechanical factor of irrigation removes the microörganisms. Not infrequently, however, microörganisms do enter these mucous membranes and produce serious infections, such as the *Treponema pallidum* of syphilis, the *M. gonorrhœæ* and the *B. chancroidæ mollis*. Sometimes these membranes are infected with ordinary pyogenic bacteria.

The Conjunctiva.—The conjunctiva is protected against infection in several ways. First, the eyebrows with their hairs and the eyelashes prevent microörganisms and particles of dust and dirt carrying microörganisms from entering the eye. Again, the lacrymal secretion or the tears flowing across the eye from the outside in serve to wash this membrane. Bacteria are frequently washed off the conjunctiva and pass down through the lacrymal duct into the nose where they meet the obstructions which have been previously discussed. In all probability the tears are only slightly antiseptic and not germicidal at all. The conjunctiva is sometimes infected with microörganisms and furthermore serves as a point of entrance into the body when it itself is not infected. There is no doubt that the *Bact. influenzæ*, the *Srept. pneumoniæ*, and other microörganisms may enter the body and get into the lymphatic and blood circulation in this way.

It is evident, therefore, that the protection afforded an individual by the body surfaces is a decided factor in the natural immunity of that individual.

The Protective Nature of Inflammatory Processes.—It has been mentioned in a previous discussion that when bacteria successfully enter a tissue and develop in that tissue a complex local change results which is designated as *inflammation.* In the majority of instances inflammation is of a beneficial nature. Fundamentally, it is always beneficial. Few examples of the pernicious results from inflammation can be given. In this connection may be mentioned the thickening of the cerebral blood-vessels in syphilis and the increase of connective tissue in cirrhosis of the liver. In these instances the inflammatory processes are brought about by the reaction of the various tissues to the irritation of the infecting microörganisms. Unluckily these reactions are not on the whole beneficial to the body, but, as before stated, inflammation is usually beneficial and may be characterized as *the reaction of tissues to injury.* The exact processes of inflammation may be traced in case an infecting microörganism succeeds in entering the tissues of the body. The organism having produced its toxic substance first causes a congestion of the blood-vessels in the region (hyperemia). Following this localized congestion there is an extravasation of plasma from the blood-vessels. This plasma immediately on leaving the vessels coagulates or clots, producing throughout the infected area fibrin and blood serum. This fibrin serves in a mechanical way to limit the infection, and it has been recently demonstrated that the fibrin possesses germicidal properties in addition. Furthermore, the serum in a large number of instances exerts a bactericidal effect upon the microörganisms. Following the extravasation of blood plasma from the capillaries, the leucocytes pass out and gather around the infected area. These leucocytes are attracted to the area due to the presence of various chemical substances (chemotaxis). They will come as close to the microörganisms as possible, depending upon the effect of the toxins which have been produced. In certain instances they will ingest the bacteria and destroy them. In such cases, the bacteria having been removed, the inflammation rapidly subsides and the infection is, therefore, checked. Such are the characteristics of an acute inflammation. Inflammations, however not infrequently depending especially upon the microörganism producing

the infection, may become chronic, and in such a case the inflammation after passing through the acute stage, as indicated above, stimulates a proliferation of the connective tissue in the part infected. In such cases, around the outside of the ring of leucocytes, which have been unable to ingest the bacteria, young embryonic connective tissue cells which are known as *round cells* are found. In case the inflammation progresses, the leucocytes are destroyed and the round cells next to the infected area assume more of an elliptical shape and are known as *epithelioid cells*. On the outside of this layer of epithelioid cells will be found newly produced round cells, and on the outside of the round cells an area of recently migrated leucocytes, those passing out in the beginning having been destroyed by the toxic action of the infecting microörganisms. Frequently the newly produced connective tissue passes on to the adult type and in this instance completely walls off the area of infection and the infecting microörganisms. In such cases the inflammation and the infection are checked. Among the diseases caused by microörganisms which have a tendency to produce chronic inflammation may be mentioned those of tuberculosis, leprosy, syphilis, actinomycosis and glanders. It is now an uncommon observation in man to note in the lungs and in other parts of the body healed areas of tubercular infection; areas that have been completely walled off by the development of adult fibrous tissue. It is probable that about ninety-five per cent of all individuals living in civilized communities are infected with *Bact. tuberculosis* some time during their lives. The inflammation produced by this microörganism passes through the acute stage and into the chronic before being successfully combated and thoroughly walled off. Such an area is known as a tubercle, and in the other diseases mentioned, similar areas of like structure are produced. It depends entirely upon the virulence of the infecting microörganisms and the resistance of the connective tissue of the individual infected as to whether healing will result.

Natural Antitoxins.—It is an observed fact that certain animals resist the action of toxins produced by bacterial and other plant and animal cells. The question arises as to whether these animals are immune to the toxins on account of the presence in their bodies of natural antitoxins or other substances. If antitoxin is present, it can be detected by experiments made by drawing off the blood serum of the animal and combining it in varying proportions with the toxin in

cstion. These experiments may be made *in vitro*. When toxins al antitoxins are combined in proper proportion and incubated t ether a non-toxic molecule is produced which when injected into a sceptible animal will produce no effect. It is, of course, necessary i his connection to inject the animal with a minimum lethal dose of t toxin in question as a control. If no natural antitoxins are present i the serum of the animal in question, the animal experimentally ir cted with the combined toxin and serum will die as a result of the n -combination of the toxin. In this way natural antitoxins may b tested. Natural antitoxins for diphtheria have been detected in t blood serum of about fifty per cent of normal humans and in about t ty per cent of horses. However, their occurrence in other animals f this specific bacterium and for other species is comparatively rare, a the explanation of the fact that certain animals are immune to t ins must be found elsewhere. It has been shown for example that t frog is immune to tetanus toxin, and that, when this animal is i cted with this toxin that a large part of the toxin remains unchanged i he circulation for a variable period of time and may be later drawn o in the blood serum and will produce a toxic effect when injected i a susceptible animal. There are no natural antitoxins present in t blood serum of the frog and it has been found that the immunity o his animal is due to the fact that there are no cells in the body p sessing the necessary side-chains (open valencies) for chemical c bination with the toxin and the subsequent intoxication of the c s does not result. It seems that the best explanation of the fact t t certain animals are immune to toxins is found in the fact that t e are no chemical substances in the cells with which toxin can c bine. It is probably not true that natural antitoxins explain all t phenomena in this connection.

Natural Antibacterial Substances.—Natural antibacterial substances a present in the blood serum and body fluids of a large number of a nals. In order to demonstrate the presence of the natural anti- b terial substances it is necessary to inject the experimental animal w a carefully washed culture of the bacteria in question. If the a nal remains uninfected, two possibilities present themselves: First, t presence of natural antitoxins; and second, the presence of anti- b terial substances. It is necessary, of course, to have excluded the p ibility of natural antitoxins, its having been demonstrated that

the organism injected produces its diseased effects by endotoxins h within the bacterial cell rather than by toxins. There is no evide indicating the presence of natural antiendotoxins in any anin The antibacterial action of the blood may be due to two constitue namely, cellular substances (leucocytes) and chemical substances the serum. The rat and the dog are both immune to anthrax but immunity of the dog is not due to antibacterial substances but to phagocytic activity of the leucocytes, while in the rat the immunit not due to the leucocytes but to the antibacterial substances. order to demonstrate the fact that the leucocytes are not respons for the immunity in the given animal it is necessary to combine bacteria in question with the leucocytes and serum *in vitro* and a incubation make a careful examination with these cells to see if t have taken up any bacteria.

Antibacterial action is due to two substances in the serum: Fi the thermostable substance which combines with the bacteria called *amboceptor;* and second, a thermolabile substance called a *complem* which combines with the amboceptor after this substance has combi with the bacterial cell. It is sufficient to say at this time that th substances occur in normal sera and that the result of their combinat with the bacterial cell causes the death of the bacteria and in some c a lysis (solution) of the bacteria in addition.

There may be present in the blood of animals antibacterial s stances of three kinds: First, those just killing bacteria (bactericid second, those killing the bacteria and dissolving them (bacteriolyt and third, the leucocytes which are active in the ingestion of the spec microörganisms. In all probability the overactivity of leucocyte every case of natural phagocytic immunity is due to the presence normal opsonins—substances which sensitize the bacteria and ren them susceptible to phagocytosis.

Normal Hemolysins.—Normal hemolysins (hemoglobin-liberat substances) are present in the serums of certain animals for the red bl corpuscles of other animals of different species, and for the same spec but never for the red corpuscles of the animal from which the serum obtained. Such substances known respectively as *heterolysins* *isolysins* and if the latter occurred the name *autolysin* would be appl

Normal Agglutinins.—Normal agglutinins for various bacteria, s as *B. typhosus, Msp. comma, Bact. dysenteriæ, B. coli,* and *Ps. pyocya*

are present in the blood serum of some animals. It is necessary, of course, to exclude normal agglutinins when testing the serum of the infected case for the purposes of diagnosis as will be mentioned later.

Normal Precipitins.—No normal precipitins for bacteria occur in the serums of animals. Precipitins for various blood sera, however, do occur. For example, human serum will precipitate the monkey serum, etc. These substances will be discussed in detail under acquired immunity.

Acquired Immunity.—Acquired immunity is that resistance which is acquired after having an infection or from artificial inoculation with the etiological microörganism of an infection or from inoculation with the products remaining in the body after infection, whether natural or artificial. Acquired immunity may be divided into two classes, namely, *active* and *passive*. *Active immunity* is that immunity resulting from an infection or vaccination. In it the body cells react and give rise to the formation of antibodies. When antibodies produced in active immunity are inoculated into other animals the immunity conferred is referred to as *passive immunity*.

Active Immunity.—Active immunity may be produced artificially in the following ways: By the injection of living bacteria; by the injection of bacteria of reduced virulence; by the injection of dead bacteria; by the injection of the secretory and excretory products of bacteria (toxins, etc.); by the injection of the disintegration products of bacteria liberated after the death of the cells (endotoxins); and by the injection of bacteria or bacterial products which in no way are related to the bacterium against which immunity is conferred.

As a result of the injection of living bacteria in small amounts or of bacteria of reduced virulence the body cells react and produce bactericidal substances (lysins, etc.). As a result of the injection of dead bacteria, the opsonins are increased in the blood. As a result of the injection of the secretory and excretory products of the bacteria, namely, toxins, antitoxins are produced. As a result of the injection of the disintegration products of bacteria, namely, endotoxins, bactericidal substances are produced. In cases where bacteria or bacterial products, which are in no way related to the bacterium against which immunity is conferred are injected, it is probable that bactericidal substances are produced. This condition only occurs in rare instances.

Passive Immunity.—Passive immunity may be conferred by the injection of antitoxins, and by the injection of bactericidal substances.

In this type of artificially produced acquired immunity the body cells do not react to any great extent and the injected antibodies remain practically unaltered. Various other antibodies may be injected into other animals and confer upon them passive immunity.

The principal antibodies produced in active immunity will be subsequently discussed.

The Origin and Occurrence of Antibodies

The toxic and some of the non-toxic substances of bacteria and cells from other sources when introduced into the body of a susceptible animal usually have the power to produce *antibodies*. Substances having the power of producing antibodies are known as *antigens*. Among the antibodies produced are antitoxins, bactericidal and lytic substances, opsonins, antiferments, agglutinins and precipitins. The antigenic substances for these antibodies will be discussed later. The mechanism of action of the antigen is of interest. It is supposed that the antigen can combine only with the cell which has the proper combining groups or receptors. The antigen combines in the same way that food products combine with the tissue cells. In case there is no group in the tissue cell with which the antigen can combine that tissue is naturally immune to the antigenous substances in question. If all the tissue cells in the body are in this condition then the individual may be said to be naturally immune. It occasionally occurs that certain cells of the body are not susceptible to the action of antigens at one time while at another they are susceptible. For example, the red blood corpuscles of the young chick are not affected by the lysin-toxin in spider poison while those of the adult are readily hemolyzed (hemoglobin liberated). It also occurs in rare cases that the antigen when injected into an animal whose tissue cells show no affinity for it or no proper receptors, will remain in the circulation for days and weeks without combining and producing any effect. The antigen, for example, a toxin, can be isolated from the blood in such a case in the same concentration and form as when it was injected. Some antigens have special affinities for certain tissues, as for example, tetanus toxin and nerve cells. In this case, however, the larger part of the antitoxin is produced by cells other than those of the nervous system. The production of antibodies for antigens probably occurs in the fol-

ing way; the antigenous substances combine with the cells utilizing a the available receptors, leaving none open for food and thus perverti the general metabolism of the cell. In such cases there is a regeneran of these chemical receptors by the tissue cells which more than cpensates for those with which the antigen has combined and as a esult the cell discharges them (chemical substances) free into he bly fluids.

The various antibodies are usually produced with more avidity b certain tissues than by others. Antibody formation may be of a sctly local character depending upon the point where the antigen is ected. For example, when abrin is placed in the eye, antiabrin is pduced, but only in the eye so injected. In the majority of cases the aibodies are produced in some special tissue or tissues at a distance fn the point of injection.

Following the injection of an antigen into the body of an animal tre is always a decrease in the resistance of that body and a decrease inhe antibodies produced followed in a short time by a marked increase inheir formation. The former condition is spoken of as the "*negative pse*" and the latter as the "*positive phase.*"

Antibodies may be transferred from mother to young before birth, b only after fetal circulation is established. It has been positively dnonstrated that antibodies are not transferred by the ovum or the sermatozoön directly. They are only carried from the blood of the mher and diffused through the placenta into the blood of the fetus. Ias, however, been shown that the eggs of immunized chickens con- ta antibodies occasionally. This is "germ-cell transmission" and n true hereditary transmission. The transferred immunity or anti- bies do not remain over two or three months in the bodies of the o pring after birth.

Antitoxins.—Antitoxins are so called because they combine with a render inert the soluble toxins. Antitoxins are produced for all th bacteria producing soluble toxins and for the toxic substances of a la e number of other plant and animal cells. Antitoxins are the free ch mical receptors of certain of the cells of the body. That is, they are ch mical substances which have been thrown off from the cells of the b y and in all probability were normally used for the purpose of taking u food substances. These chemical substances are produced in excess o hose actually needed by the cell due to a stimulation of the cells by

the toxin. The antitoxins are labile substances which cannot be analyzed. They may be similar to euglobulins. They are composed of molecules of large size. Antitoxins when present in the body of an animal are protective and in many cases curative. According to Ehrlich, toxins are assumed to possess two chemical combining groups, one known as the *haptophore group* which combines with the cells and another known as the *toxophore* group which combines with the cell after the haptophore group has combined and this produces an intoxication of the cell. The haptophore group of the toxin molecule is thermostable (heat resistant) and the toxophore group is thermolabile (heat susceptible). When a toxin is injected into the body of an animal, or is produced during the process of an infection, the haptophore group combines with the cells with which it has an especial affinity and with receptors (chemical substances which are unsaturated and open to combination with other chemical substances) of these cells. The chemical receptors of the cells with which the toxin-haptophore group combines are designated as *haptophile receptors*. It is probable also that the toxophore group of the toxin combines with other chemical receptors in the cell after the haptophore and haptophile groups have combined. These are designated as *toxophile receptors*. The haptophore receptors of the toxin having combined with the haptophile receptors of the cell, the toxophore group of the toxin then combines and intoxicates, stimulates or sometimes kills the cells depending on the affinity for the cells and the concentration of this group. In case the cell is not killed it is stimulated and begins after a time to return to its normal function. All the available receptors of the cells having been occupied and combined with, the cell sets about to generate new chemical receptors in order that food substances and other chemical substances may be taken up. The cells produce these haptophile receptors in excess, that is, there is over-compensation, and they are subsequently excreted into the lymph and blood. These haptophile receptors are in fact the chemical substances which we know as *antitoxins*. It is not only the cells which both the haptophore and toxophore groups of the toxin combine because of special affinity, which make all the antitoxin, but cells which are widely separated from those which have an especial affinity for the toxin, also produce antitoxin. For example, tetanus toxin has especial affinity for nerve tissue but this tissue produces little of the antitoxin. In this case most of the antitoxin seems to be produced

th spleen, lymph glands and bone marrow. The haptophore groups of e toxin have at least combined with these cells and stimulated them to he overproduction of haptophile receptors.

t has been mentioned that the antitoxins are *protective* to the body in cted. The haptophile receptors (antitoxins) before they are thrown of combine with the toxin-haptophore and often the toxophore group do not have the opportunity for combining and killing the cells. This is case there is no special affinity for the cells, as in the above-men- ied chief antitoxin-producing cells in tetanus. In such cases fre- ntly all the available toxin is bound and very little is left to combine wi the tissue with which it has an especial affinity, as is the case with te nus toxin and nerve tissue. The antitoxins serve in this instance as rotective substances. Furthermore, in case the antitoxin is ex- cre d into the blood and lymph it serves in addition as a *curative* agent, all he toxin which is produced combining with all the available anti- to n in the circulation and none is left to combine with the cells of th body. The maximum affinity is always between toxin and anti- to n rather than between toxin and cell, if there is any antitoxin pr ent. Antitoxins are prepared artificially and used for both pro- ph actic and curative purposes in the treatment and prevention of ce ain of the infectious diseases such as tetanus and diphtheria.

ntitoxins are also produced in the bodies of animals which are to all ppearances immune to the toxins concerned. For example, the all ator is immune to tetanus but when tetanus toxin is injected into th animal tetanus antitoxin will be produced. In this case the hapto- ph e group of the toxin has combined with certain of the cells of the bo , but with such cells as give no opportunity for the toxophore gr p to combine, or have no affinity for this group. In the case of th alligator the nerve tissue seems to possess no chemical receptors for th toxin.

here are certain animals which are very susceptible to the action of rtain toxins and which will not produce antitoxin when the toxin is in ted. For example, the guinea-pig and the rabbit will not produce te nus or diphtheria antitoxin when injected with small and gradually in easing doses of tetanus or diphtheria toxin. If the toxin is modified ch nically by the addition of chemicals such as terchloride of iodine or by eat these animals may be immunized and will produce antitoxin. In his instance the virulence of the toxophore group is reduced and it

is possible to inject the animals with more toxin, thus combining w more cells and finally liberating more antitoxin.

It should also be noted that animals of the same species vary their power to produce antitoxin. The production of the prod varies with the age and general condition of the animal and with t duration and the degree of toxicity of the toxin used. On accou of this condition it is necessary to establish units or standards determining the strength of antitoxins.

As stated in the discussion of natural immunity to toxins, th are some animals which when injected with toxins do not possess c which have receptors open for chemical combination and as a res the toxin remains free in the circulation for varying periods of tin For example, as before stated, the frog is immune to tetanus and injection of toxin will not produce any antitoxin. If tetanus to is injected into this animal it will remain in the circulation in t same form as injected and can be withdrawn after a few weeks o month.

The Mechanism of the Neutralization of Toxin by Antitoxin.— At one time it was supposed that the antitoxin was but a toxin ir little different form but this has been absolutely disproven. T amount of antitoxin produced is much greater than the amount toxin which is injected or produced during an infection.

The union between toxin and antitoxin is of a definite chemi nature. After these two substances unite the resulting compound absolutely harmless and differs from both the toxin and the antito in that it is much more stable.

In the beginning all experiments dealing with the union of to and antitoxin were performed in the body of an experimental anim (*in vivo*) but finally Ehrlich showed that they would act and comb equally well in the test-tube (*in vitro*) and could be studied much m easily.

The various toxins are neutralized by their antitoxins with varyi rapidity. The concentration of these chemical substances, the te perature, the character of the medium in which they are placed, and t amount of electrolytic salts present, are accountable for the differen in length of time of combination. In the main these substances a like most chemicals and some of them show evidences of following t laws of multiple proportions. As a matter of fact the same laws wh

overn the union of toxin and antitoxin govern other antibodies and heir antigens.

As before stated, toxins have a greater affinity for the free haptophile receptors of cells (free antitoxin) than for those still associated with the cells. Toxin and antitoxin will always combine, if the opportunity presents itself, before toxin and body cells will enter into chemical union. Furthermore, in certain instances, such as in diphtheria, when the toxin has been partially bound by the body cells and antitoxin is produced in sufficient quantities or is injected, the toxin-cell chemical union will be broken up and the toxin and antitoxin will combine. Obviously, antitoxins of this kind are very valuable in effecting a cure in certain infections. In the above-mentioned cases, the union between the toxin and the cell is comparatively unstable but this is not true in every case, as for example, in tetanus or lockjaw. In this case when once the toxin is combined with the cells of the nervous system and other body cells it is very difficult to break up the chemical combination by the addition of antitoxin. It requires exceedingly large doses and these rarely act efficiently. The union between toxin and body cells in this instance is very stable. We have here an explanation why tetanus antitoxin is of so little use for therapeutic purposes. It is, however, of use as a prophylactic when free toxin is being produced in the body. Diphtheria antitoxin is efficient both as a curative and prophylactic agent for the reasons which have been discussed above.

Antitoxin like toxin is fairly unstable and such agents as heat, light, and chemicals, affect it and reduce the toxicity. It may, however, be dried and kept for long periods of time in the dark. It is necessary in the commercial preparation of antitoxin and in its experimental study to have a unit or standard of measurement.

Units of Antitoxin.—In order to arrive at a standard it is necessary to accurately test a given antitoxin to determine the number of so-called antitoxic units it contains.

In the accurate study of the neutralization of the toxin by the antitoxin it is noted that adding fractional amounts of the antitoxin to the L° dose of the toxin and injecting the resulting mixture into test animals (guinea-pigs), there is not a corresponding decrease in the toxicity as would be expected. The toxin is made up of various parts. The part just mentioned has a great affinity for the antitoxin but is not

really toxic. Such parts of the toxin molecule are called *protoxoids*. The protoxoids compose about one-fourth of the amount of toxin necessary to saturate one immunity unit. After the one-fourth antitoxin is added to the L° dose of toxin the mixtures of toxin-antitoxin become less toxic for the experimental animals down to the point where approximately three-fourths of the amount of toxin necessary to saturate one unit of antitoxin has been used (three-fourths of L° dose). This fraction is *true toxin*. The toxicity of the mixture does not decline from this point when antitoxin is added up to one immunity unit, and it has been demonstrated by Ehrlich and others that this is due to another part of the toxin molecule which has a lesser affinity for the antitoxin than the true toxin itself and the protoxoid. This part of the molecule is called an *epitoxoid, true toxoid,* or *toxon*. The toxin molecule necessary to saturate one unit of antitoxin is, therefore, made up of *one-fourth protoxoid, one-half true toxin, and one-fourth epitoxoid, true toxoid* or *toxon*. The toxon is in certain instances slightly toxic and is supposed by some to be a secondary toxin and in certain diseases such as diphtheria, this substance has a weak affinity for antitoxin and is a possible cause of diphtheritic paralysis.

Antitoxins may be prepared for all the bacteria producing soluble toxins, such as *Bact. diphtheriæ, B. tetani, B. botulinus* and *Ps. pyocyanea*. Antitoxic substances may also be made for some of the products of other bacteria such as the *Strept. pyogenes* but these differ from true antitoxins. Antitoxins may also be prepared for the toxins of certain plant cells, such as abrin, reçin, crotin, and for the toxins of animals such as snake venom and spider poison. These substances are in the main similar to those produced by bacteria, although in certain characteristics they differ materially.

Lysins and Bactericidal Substances.—Under the lysins will be discussed those substances occurring in normal and immune sera which have the power of destroying and disintegrating bacteria, those disintegrating and liberating the hemoglobin of erythrocytes (red blood corpuscles) and those substances which have a lytic action on various body cells. The substances which act on the bacteria are called *bacteriolysins*, those acting on erythrocytes are called *hemolysins* and those acting on the other body cells are called *cytolysins*. The mechanism of these lytic processes is quite complex. It should also be noted in this connection that there are certain substances which

ll or seriously injure bacteria and body cells and do not actually integrate them. Such chemical bodies are designated respectively *bactericidal substances* and *cytotoxins*.

The first observations in regard to bactericidal and bacteriolytic bstances were made by Nuttall and later by Büchner. Büchner ted these substances in normal serums and other body fluids and med them *alexins* (Gr. to guard). He assumed that they were ncerned in the immunity of the body. This is not necessarily true certain blood serums are frequently highly bactericidal when the in-idual is relatively susceptible. This is true of human blood serum d *B. typhosus*. Furthermore, in certain instances the animal is mune to the disease and the serum is not in any sense bactericidal. is is the case with the dog and *Bact. anthracis*.

Pfeiffer a number of years ago observed that when *Msp. comma* of iatic cholera was introduced into the peritoneal cavity of the normal inea-pig that the bacteria underwent lysis. He also noted that the ocess was much more rapid in the immune guinea-pig. Pfeiffer had e idea in the beginning that lysis did not take place anywhere but in e body of the animal but later it was demonstrated by a number of n, among them Metchnikoff, that the lytic action would also take ce in the test-tube (*in vitro*).

Bordét and others later showed that some normal serums possess e power of liberating the hemoglobin in red blood corpuscles. It s also shown that these hemolytic substances could be developed in e body of an animal if that animal were injected or immunized with suspension of erythrocytes. The phenomenon of hemolysis is easily served and studied and the amount of the hemolytic agent can be curately determined as the amount of hemoglobin liberated varies cordingly. The mechanism of hemolysis and bacteriolysis corre-ond exactly and accordingly much about the latter process was first rked out by experimentation with hemolysins.

Lytic substances can be prepared for a large number of bacteria and many body cells, as before stated. These substances may be rkedly increased by the usual processes of immunization. Those bstances which have the power to produce lysins are called *lysinogens* d are distinct antigens. The lysins are antibodies. The lysins may be pared by injecting the experimental animal with the live cells, the

dead cells, the disintegration products of cells and in some cases with th metabolic products of cells.

The Structure of Lysins.—Lysins and bactericidal substances hav been shown to be composed of two distinct parts: one a thermolabi part known as the *complement* which is destroyed at a temperature 55° to 60° for thirty minutes; and another part which is therm stable, known, on account of its double combining ability, as an *amboce tor*. This amboceptor will withstand heating to 60° for twenty-fo hours but if the temperature is raised to 70° it is readily destroyed. kept at ordinary room temperature or in the ice box amboceptors wi remain active for years. According to Ehrlich, amboceptors are th free chemical receptors of the body cells. They are produced in th same way as antitoxins but differ from these bodies in that they hav two combining groups, one known as the *cytophile* group with whic the amboceptor combines with the bacteria or other cells, and the oth known as the *complementophile* group, with which it combines wit the complement. The complement seems to be a normal constitue of the blood serum and other body fluids. It is undoubtedly produce by the various body cells (leucocytes et. al) and during the immuniz tion of animals with certain antigens it is probably increased on slightly, if any, in amount. The complement is supposed to be con posed of two groups also, one a haptophore with which it combin with the amboceptor, and another a zymophore which readily produc the lytic action after the haptophore has combined with the amboce tor. On heating the complement the zymophore group is destroye and a *complementoid* is produced. This substance is similar to a toxic and will combine with amboceptor but no lysis will result. It i however, the amboceptor, or so-called immune body, that undergo the decided increase during the processes of immunization. It can accurately demonstrated that the amboceptor must combine with th cell in question before the complement can combine. Cells, such bacteria or erythrocytes, may be saturated with amboceptor ar washed and when the complement is added and combined, lysis tak place. The complement will not combine with the cells under ar circumstances unless amboceptor is present and has first combine with the cells. It is probable in a given serum or body fluid that the are several complements which may activate a variety of amboceptor

However, it has been shown that the same complement will activate variety of amboceptors of certain kinds.

While the majority of lytic serums are thermolabile some have been oted which are thermostable to a certain degree. Hamilton has described such a serum resulting after immunizing animals to *Bact. seudodiphtheriæ* and Horton has noted thermostable substances in ormal rat serum which are lytic for *Bact. anthracis*.

Various serums have been noted which possess amboceptors for ertain cells but are not lytic because they do not possess the necessary omplement. For example, the serum of the dog contains amboceptors or *Bact. anthracis* but no complement. If in this instance a foreign omplement such as that in guinea-pig or rabbit serum is added there ill-be lysis of the bacterial cells.

Occasionally the absence of complement is of benefit to the animal n question and may account for the seeming natural immunity. For xample, the venoms of the poisonous snakes are nothing more than mboceptors and when these substances are injected into an animal ody such as a hog, which does not possess the required complement, o lysis of the body cells takes place. On the other hand, should the nimal, such as a rabbit or man, possess the necessary complement, as ney do, lysis will take place.

Substances are sometimes present normally in serums which have ne power of combining with the amboceptors which may be present, nd prevent the latter from combining with the cells so that when the omplement is added there will be no lysis. Such substances must be esignated as *antiamboceptors*. These antiamboceptors (*antiantibodies*) ay be developed in an animal by immunization with amboceptors definite kinds. There are other substances which may also engage ne amboceptors which cannot be called amboceptors in the true sense ut they accomplish the same purpose and are, therefore, classed with ese bodies.

The Deviation of the Complement.—The complement may be deviated several ways and as a result lysis of the cells in question may be revented.

Occasionally there is noted in serums normally substances which ay combine with the complement and prevent this body from comning with the amboceptor. Such substances are called *anticomplements* and may be produced artificially by the immunization of animals

with complement. Occasionally complement is absorbed by tissue cells and prevented from combining with amboceptor. In case there is an excess of amboceptors in a serum and only a small amount of complement, it may be deviated. In this case the cells will have taken up all the possible amboceptor and there will be an abundance of amboceptor free in the serum. It has been demonstrated that complement will combine with free amboceptor before it will combine with the amboceptor which has been bound to the cells. In this case all the available complement will be taken up by the amboceptor which is free and consequently there will be no lysis. This fact is of importance in certain infections where the development of bacteriolytic substances are of importance and necessary in effecting a recovery. The infectious microörganisms may not be destroyed for the above reason.

The Deflection of the Complement as a Test for Antibodies.—A very ingenious procedure has been devised for the testing of serums for unknown antibodies similar to bactericidal substances and lysins. The method of demonstrating the fixation of the complement was first worked out by Bodét and Gengou. The reaction is made use of in the test for syphilis which is briefly stated as follows: when the syphilitic antigen is combined with the supposed amboceptor in the blood serum of the suspected case of syphilis and a foreign complement, which has been accurately standardized, is added, this complement is bound and is therefore, prevented from combining with red blood corpuscles, and a hemolytic amboceptor which may be added later. Hemolysis is therefore, prevented. The technic of the test is as follows: the syphilitic antigen is prepared by making an aqueous or alcoholic extract of the liver of syphilitic fetus or in several other ways. This antigen is supposed to contain the protein products of the *Treponema pallidum* the etiological microörganism of syphilis. The blood serum of the suspected case of syphilis is heated to 56° for thirty minutes in order that the normal or immune serum complement may be destroyed. The new complement is supplied from normal guinea-pig serum. Before beginning the test it is necessary to have a rabbit immunized with some hemolytic antigen, such as sheep erythrocytes. There is developed in the serum of the rabbit the hemolysin for sheep corpuscles which when combined with these corpuscles will cause a liberation of hemoglobin. In the rabbit serum there are both hemolytic amboceptors and complement. It is necessary to heat this hemolytic rabbit serum to 56° for

hirty minutes in order to destroy its complement and also it is necessary o accurately find out the amount of guinea-pig serum which will com- lement the resulting hemolytic amboceptor. This definite amount of omplement having been determined, it is mixed with syphilitic antigen lus the syphilitic amboceptor, mentioned above, and allowed to incu- ate for one hour and thirty minutes at 37°. If the serum is from a ase of syphilis the antibodies (amboceptors) will be present and com- ine with the antigen, and also the guinea-pig serum complement. The ext step in the technic is to add to the above-mentioned mixture the emolytic amboceptor and its antigen, sheep corpuscles. If the com- lement has been bound there will be none left to combine with the emolytic amboceptor and no hemolysis of the sheep corpuscles will esult. If the patient's serum does not contain syphilitic amboceptors r antibodies, the complement will not be bound and hemolysis will esult. This test has been designated as the *Wassermann test* on ccount of the man first working it out in the case of syphilis, and has hown itself to be very efficient in the diagnosis of this disease in uspected cases. Many modifications of this test have been devised, ome of which are very accurate.

The fixation of the complement may be made use of in the detection f any bacterial antibody, the procedure being approximately the same s above indicated and the hemolytic system used as an indicator as in he case of syphilis. The antigen, however, is different. When working ith specific bacteria a suspension of bacterial cells in 0.85 per cent odium chloride solution constitutes the antigen.

Cytotoxins and Cytolysins.—The names *cytotoxin* and *cytolysin* are sed synonymously and are applied to those substances in serums and ther body fluids which have the power of destroying cells other than rythrocytes. In a broad sense any substance destroying a cell would e cytotoxic but the terms are usually applied in the more limited anner, as above indicated.

Cytotoxins are produced in the same manner as other antibodies. he immunization of an animal, for example, with renal (kidney) cells, roduces in the blood serum of that animal a cytotoxin for the paren- hymatous cells of the kidney. Cytotoxins can be produced for prac- ically all the parenchymatous cells of the body. These immune bodies re not very specific and even careful experimentation leads to confusing esults. For example, when an animal is immunized to kidney cells here is produced in the body of the immune animal cytotoxins for

kidney cells and also cytotoxins in smaller amounts for other paren chymatous cells such as those of the liver. In the beginning it was sup posed that the cytotoxins would be of value in the study of the physic logical functions of organs and tissues. For example, a cytotoxi having been produced for the thyroid gland or adrenal gland it would b possible to inject this into another animal, destroy the gland, and the note the effect on the body. It was thought it might be possible t produce anticytotoxins which would be able to counteract the action o those cytotoxic substances which are produced in the body during th course of infections. However, the lack of specificity of the cytotoxi renders these procedures only theoretically possible. The fact tha cytotoxins are produced for cells other than those used in the process o immunization indicates that there are similar chemical substances in th various cells.

There are *autocytotoxins* produced in the body. These probabl result from the absorption of the products of disintegrated tissue cells If no *anticytotoxins* for these autocytotoxins are produced, or they ar not destroyed in some way, a very "vicious cycle" would result i that more of the specific cells of the organ or tissue used would be de stroyed. Cytotoxins have been prepared for leucocytes and thes substances are sometimes developed during the progress of an in fection. The *leucocytotoxins* have perhaps been studied more tha any one of others.

When ova are used for the purpose of producing cytotoxins, beside producing these substances in the serum of the immune animal cytotoxins for spermatozoa of the same species are also produced showing that these cells have some chemical substances in common.

Metschnikoff, following his idea that old age is due to a destructio of tissue by the mononuclear leucocytes, hope that it would be possibl to produce a cytotoxin for these cells. It is claimed by some that ther are specific substances produced by the exhaustion of certain cells that is, a toxin of fatigue. Weichardt has produced an antibody fo this toxic substance which must in reality be an anticytotoxin.

It has been suggested that the cardiac hypertrophy in nephritis i due to the effect of a nephrocytotoxin on the peripheral blood-vessel causing increased diastolic pressure on the heart.

Another interesting substance has been produced and this is calle syncytiolysin. It is prepared by immunizing animals with placenta

lls. It is claimed that this cytotoxin produces on injection symptoms milar to those noted in eclampsia and it has been suggested that the oduction of such a body in the pregnant woman from the placental lls may be the cause of this serious condition. Liepmann claims to ve demonstrated placental constituents in the blood of pregnant omen by means of the precipitin test. These bodies must be the ntigen of cytotoxins. He states that when the blood of the pregnant oman is mixed with the specific syncytiolysin produced by im- unizing an animal with human placenta that a precipitate occurs. e suggestst he possibility of a serum test for pregnancy. Abderhalden s reported some interesting results with the serum test for preg- ncy and cancer. His findings cannot, however, be regarded as nclusive.

Cytotoxins are similar to bacteriolysins and hemolysins. They nsist of amboceptors which are activated by the complement which is rmally present in the serum or other body fluids.

The Opsonins and Phagocytosis.—It was shown a number of ars ago that certain types of leucocytes and other body cells were pable of ingesting bacteria and other plant and animal cells. The echanism of this process was not known until Wright and Douglas monstrated certain substances in the blood serum and other body uids which have the power of rendering the bacteria susceptible to agocytosis. These substances are known as *opsonins* (Gr. I pre- are food for). The phenomena of the phagocytosis depend almost holly on these special opsonins. Leucocytes which have been washed ee from all serum will not take up bacteria except a few in rare in- ances. Bacteria which have been placed in contact with blood serum other body fluids may be thoroughly washed, and then when they e placed in contact with the leucocytes, they will be taken up. The sonin reacts chemically with certain substances within the bacteria, d so to speak, sensitizes them. Opsonins are present in many normal rums for the various bacteria. They may be produced in animals t containing them by the process of immunization with various ntigenous microörganisms. Opsonins are destroyed at about 60° r thirty minutes, but there is some variation among them. When pt at 0° opsonins will remain active for several days, but at a mperature of the body, 37°, after the serum has been withdrawn, ey rapidly deteriorate. Many opsonins have the structure of

agglutinins and precipitins, although they bear some points of r semblance to antitoxins and complements. They possess two s called chemical groups, a "combining group" by which they enter in chemical union with the bacteria and a "functional group" whi really sensitizes the microörganism and makes it phagocytable.

It has been shown that the opsonins may be increased in t serum of the animal or injected individual by the injection of heat (60°) cultures of the specific etiological microörganisms. Such su stances are called *opsonogens* or *vaccines* (bacterins). Vaccines a used to a certain extent in the treatment of the various pus infectio due to the staphylococci and also in tuberculosis and pneumonia. is supposed that the opsonins are produced in the subcutaneous tissu and in the muscles.

The Opsonic Index.—The concentration of the opsonins may l recorded in an individual in the following ways. Suppose the le cocytes of the infected individual take up a certain number of bacteri say an average of 5, after counting 50 to 100 polymorphonuclear le cocytes. In this case the *phagocytic index* is said to be 5. Agai suppose the leucocytes of the normal individual take up 15 of the ba teria in question, the average after counting 50 to 100 leucocyt being always taken. The phagocytic index in this case would be 1 In order to determine the *opsonic index* of an infected individual t phagocytic index of the normal individual is taken as a denomina of a fraction and the phagocytic index of the infected individual as t numerator of the fraction. In the above illustration this would b $\frac{5}{15}$, $\frac{1}{3}$ or reduced to decimals 0.33+. The opsonic index, it can b seen, is somewhat of an indication of the resistance of the particul individual to the infecting microörganism in question. By the us of vaccines the opsonic index may be raised to at least 1.0 or eve more, showing that the leucocytes are actively phagocytic and th opsonins increased in concentration in the blood serum. In such case recovery would be indicated. When vaccines are injected in th treatment of infections the opsonic index has been shown to vary fro time to time. Within a few hours after the injection the opsoni index falls below what it was at the time of the injection. This lowe ing of the index is known as the "*negative phase.*" Following th fall in the index there is a continuous rise to a point equal to what i was in the beginning and above this point. This rise in the opsoni

dex is known as the "*positive phase.*" The individual receiving the accine usually shows an increase in the symptoms during the "*negative phase.*" Obviously, it is necessary not to give a subsequent injection of vaccine until the patient is at the height of the "*positive phase.*" This can be best determined by determining the opsonic dex.

Occasionally counts are made of the number of leucocytes which are tually taking up bacteria, disregarding the number of bacteria within e cells. The determination is always made on the basis of 100 and e per cent of leucocytes which are phagocytic is taken as the so-called *rcentage index*. The percentage index also gives an idea of the restance of the individual. It has been shown that in the practical ork of treating infections with vaccines it is not absolutely necessary determine the opsonic index or percentage index. The positive d negative phase may be determined fairly well by general clinical servations on the infected individual. Virulent bacteria are not adily phagocytized. For example, virulent streptococci and pneumococci are not phagocytized as easily as non-virulent forms. It ems in this instance that there is some toxic or poisonous substance oduced by the bacteria that is antagonistic to the opsonins or perhaps antiopsonin is formed.

The presence of opsonins in the body fluids of an animal is not solute proof that such animal is highly resistant to infections. The sistance really depends on the activity of the phagocytes and in rtain cases where the opsonins are high in concentration the phagocytes are not active. In other cases the reverse is true and in these ses opsonins and phagocytosis are of the utmost importance in the immunity of individuals. For example, in anthrax the immunity of the dog is due to opsonins and phagocytosis, while in the rat, although opsonins are present, there is no phagocytosis and immunity is due to antibacterial substances in the blood serum. In certain infections, such as typhoid fever, influenza, and uncomplicated miliary tuberculosis, there is a deficiency in leucocytes (leucopenia) and consequently even if the opsonins were concentrated and the bacteria sensitized there would be very little increase in the immunity from these causes.

Hemoöpsonins.—It has been demonstrated that very frequently opsonins for red corpuscles are present in the sera and body fluids of animals. Such bodies sensitize the red blood corpuscles and render

them susceptible to phagocytosis by the polymorphonuclear leucocyt and the epithelial and other body cells. They are designated as *hem öpsonins*. Occasionally *iso-* and *autohemoöpsonins* are present normal sera. For example, in human blood serum, it is probable th the process of red blood corpuscle destruction which takes place in t spleen may be referred to the action of these types of opsonins ar various phagocytic cells.

AGGLUTININS.—Agglutinins are substances, present in the blo serums and body fluids of normal and immune animals, whi have the power of producing a clumping and sedimentation of t microörganisms causing the specific infection or used in artificial ir munization. The relationship of the agglutinins to the phenomena immunity and the other antibodies which are produced during t process of infection and experimental inoculation is not known. O of the first agglutinins to be observed was that occurring in the blo serum in cases of typhoid fever and the agglutination reaction is n made use of in the diagnosis of this disease (*Widal test*). Agglutini are specific substances and at high dilutions only cause a clumping the microörganisms which give rise to their formation (antigens).

Normal Agglutinins.—Agglutinating substances, as above stated, a frequently found in normal serums. In this case no direct connecti between their formation and specific microörganisms can be establishe Normal human serum frequently contains agglutinins for *B. typhosu B. coli*, *Bact. dysenteriæ*, and occasionally *M. pyogenes var. aure* and *Msp. comma* in certain rare cases. Agglutinins for *B. typhos* which are present normally in the serum may give rise to confusi when this test is used for the diagnosis of typhoid fever. It is, the fore, necessary to dilute the serum of a suspected case of typhoid fe at least one to forty or one to fifty times in order to exclude the norm agglutinins and the so-called coagglutinins.

The Production of Agglutinins.—Agglutinins may be produced ar ficially by the injection of bacteria, dead or alive, into the veins, s cutaneous tissues or peritoneal cavity. In rare cases they may be p duced by feeding the bacteria, injecting them into the air passages the lungs or by rubbing them into the skin. It is probable that t highest concentration of agglutinins results from the injection of de bacteria. It is, however, necessary that these bacteria be not subject to a temperature above 62°. Many pathogenic and non-pathoge

cteria form agglutinins when injected into the body. The concentration of the agglutinins produced varies greatly. Very high agglutinating serums are noted, such as, for example, one in one million when *typhosus* is used and one in two million when *Msp. comma* of *Asiatic cholera* is used. Often two strains of the same organism will vary greatly in their power to produce agglutinins. Again, the concentration of the agglutinins in an infected animal varies from day to day, and in order to make an accurate observation it is necessary to make repeated examinations on subsequent days. For example, in typhoid fever the agglutinins one day may be thirty times as strong as on a subsequent day.

The Distribution of Agglutinins in the Blood.—As before stated, these antibodies are found in practically all the body fluids. They reach their highest concentration in all probability in the blood serum. In certain cases they are in high concentration in the milk. Agglutinins are also present at times in the sputum, tears, and the humors of the eye.

Inherited Agglutinins.—Agglutinating substances may be transferred from the mother to the offspring *in utero*. It has been frequently demonstrated, for example, that the offspring of mothers who have recently recovered from typhoid fever or who are infected at the time of the birth, have agglutinins in the body fluids. The same is true of the offspring of glandered horses. Notwithstanding the fact that the milk is frequently rich in agglutinins, these substances are not transfered to the offspring to any great extent by this means.

The Substances Concerned in Agglutination.—There are two distinct substances concerned in this reaction, one substance which is present in the serum or body fluids of the infected or immune individual, and other substances which are present in the microörganisms which are agglutinated. The substance in the serum, as before stated, is known as the *agglutinin;* the substance (antigen) in the bacteria or other microörganisms is known as the *agglutinogen*. When agglutinins and agglutinogens are combined together a new substance is formed which is designated as an *agglutinate*. As to the location within the bacterial cell of this agglutinogen (agglutinum) there is some dispute. Various authorities have stated that it is present in the cell wall or on the cell wall. Others have held the view that it is located within the cell protoplasm and in certain instances in the flagella. Without doubt, in certain cases this substance is excreted from the cell into the surround-

ing medium, as is shown by the fact that when filtrates of bacterial c
tures are injected they frequently give rise to the formation of agg
tinins. This agglutinogenic substance is specific and varies with t
species. There are, however, very closely related substances of th
character among some groups of bacteria. When these agglutinoge
substances are injected into the animal they frequently give rise
agglutinins which when combined with other members of this gro
will produce agglutination in low dilutions. Such a reaction and pr
erty is known as "*group agglutination*," and the agglutinins produc
in such a case are known as *coagglutinins*. For example, the serum
the patient suffering from typhoid fever or of an animal immuniz
with *B. typhosus* will produce an agglutination first of *B. typhosus*, b
in addition, an agglutination of *B. coli*, *B. paracoli*, *B. paratyphos*
and *B. enteriditis*. The agglutination of these last-named organisr
of course, will not be active except in low dilutions, and in order
exclude them satisfactorily it is necessary to dilute the serum to
higher point. This phenomena of coagglutination is due to the fa
that there are some chemical substances (agglutinogenic) within th
bacteria which are common to all and which give rise to the formati
of agglutinins, which are chemically similar to each other in certa
respects.

Structure of Agglutinins and Agglutinogens.—According to Ehrlic
conception the agglutinins are composed of two chemical groups
haptophile or combining group with which it combines with the hap
phore group of the agglutinogen and a zymophorous or agglutinoph
ous group which actually produces the agglutination. The agglutin
gen is also composed of a combining group known as the haptoph
group with which it combines with haptophile of the agglutinin. It
probable that this same haptophore group will combine also with va
ous tissue cells and give rise to formations of agglutinins which are rea
free haptophile receptors of the tissue cells which have been acted up
by the agglutinogenic substance contained in the bacteria.

Agglutinoids.—It is possible by means of heat and chemicals
destroy the zymophorous group of the agglutinin leaving only the hap
phile group. Such a substance is known as an *agglutinoid*, being simi
to a toxoid. A temperature of not to exceed 60° to 70° is necessa
to produce this substance. Agglutinoids will combine with the agg
tinogen of the bacteria but they will not produce a clumping or an *agg*

tate. Occasionally in some fresh serums substances are found which ve a greater affinity for the agglutinogen of the bacteria than the aglutinins have. Such substances are designated as *proagglutinoids* ad are in this respect similar to protoxoids.

The Stages of Agglutination.—There are two distinct stages of the aglutination reaction. Neither of these stages can take place unless sne salts or electrolytes are present. Sodium chloride is the common st present. The first phase of the agglutination reaction is a union bween the agglutinin and the agglutinogen of the bacteria. The sond phase is the actual clumping of the bacteria. It is supposed that in this last phase the zymogenic group of the agglutinin is acting. I the first phase the haptophile group of the agglutinin is combined wh the haptophore group of the agglutinogen.

There are some bacteria that cannot be agglutinated, as for example, *Bt. pneumoniæ* of Friedlander, and in rare instances *B. typhosus* cnot be agglutinated. It is possible, for example, to grow *B. typhosus* aa temperature of 42° and cause it to lose its power of producing aglutinins. Bacteria may also be modified chemically so that they wl lose the power to produce agglutinins.

Agglutinins bear no relationship to bactericidal substances, antitins, opsonins or any of the other antibodies. They are both of use in he determination of species of bacteria when a known agglutinating sum is used, and they are also of use in determining the cause of inctions where a known culture or agglutinogenic substance is used. Te agglutination reaction is used in the diagnosis of typhoid fever, patyphoid fever, glanders and dysentery.

Hemoagglutinins.—Agglutinating substances are sometimes prodced for red blood corpuscles when these cells are used in the immunation of an animal. Such agglutinins when combined with the cpuscles produce a clumping which is known as hemoagglutinatin. The mechanism of the reaction is the same as that of bacterial aglutinins. It is possible that hemoagglutination is one important factor in the production of agglutination thrombi in certain inctious diseases such as typhoid fever.

Precipitins.—Another group of substances, which are antibodies, isproduced through the processes of immunization which have not bn definitely connected with the phenomena of immunity. These sustances are known as the *precipitins.* Precipitins may be produced

for the protein substances of most bacterial cells and a large varie of other plant and animal cells, such as blood serum, milk and grai They were first demonstrated in 1897 by Kraus, who noted that t bouillon filtrates of cultures of *B. typhosus*, *Bact. pestis*, and *M. comma* would cause precipitates when mixed with the blood serum tak from cases of these diseases. The precipitin reaction is definite a specific. The protein substance used in immunization or concern in the infection is the only one which is precipitated when the an serum is added. To the protein substance which produces the preci tins the name *precipitinogen* is applied. To that substance in the blo serum and body fluids of the immunized or infected animal or pers the name *precipitin* is applied. The combination between the preci tinogen and the precipitin forms a new chemical substance known a *precipitate*. Precipitin may be formed in various parts of the bo for example, in the parenchymatous cells of the organs and by t leucocytes. *Bact. diphtheriæ* will not act as a precipitinogen a will not produce precipitins. This is practically the only bacteri which will not yield these antibodies.

Normal Precipitins.—Precipitins for alien blood serums have be found in the organs and blood of seemingly normal animals. Norn precipitins for bacterial proteins have not been demonstrated to certainty.

Mechanism of the Formation of Precipitins.—The mechanis of the formation of precipitins is similar to that of other antibodi When the precipitinogen is injected into the body of an animal, combines with certain of the body cells, occupying chemical recept which otherwise would be used for the taking up of food produc As a result the cells produce new receptors and the number of th more than compensate for the ones already utilized. The chemi receptors are finally thrown off into the body fluids and form the p cipitins. It is supposed that the precipitinogen contains haptoph receptors which combine with the haptophile receptors of the cel When these haptophile receptors are regenerated and produced excess, as before stated, they are thrown off into the body fluids and a really what we know as precipitins. Precipitins are produced m commonly for widely different or heterologous substances or seru (heteroprecipitins).

Autoprecipitins and Isoprecipitins.—It has been demonstrat

hat animals will not produce precipitins for their own protein sub-
tances. For example, if an animal is bled and injected with its own
lood serum an antibody will not be produced. Therefore, *autopre-
ipitins* do not occur. Again it has been shown that only in rare
istances do animals produce precipitins for members of the same spe-
es. For example, if an animal, such as a goat, is bled and the blood
erum injected into another goat, it is only in rare cases that the second
oat will produce an antibody which is capable of producing precipi-
ation of the proteins in the first goat's blood serum. Such precipitins
re known as *isoprecipitins* and occur only in a very small per cent of
ases and with no regularity.

The Phenomena of Specific Inhibition.—When precipitins are heated
o low temperature (50° to 60°) or are subjected to the action of
ght or certain chemicals, their power to produce a precipitate when
mbined with a precipitinogen is destroyed. The precipitin which
as been heated becomes a *precipitoid* similar to an agglutinoid or a
xoid. Their ability to combine with the precipitinogen still remains.
is possible, therefore, for precipitoids to combine with all the avail-
le precipitinogen so that when fresh precipitin is added no precipitate
ill occur. This is known as *specific inhibition* and sometimes leads
very confusing findings in the study of these immune bodies.

Antiprecipitins.—When an animal has produced a precipitin in its
ood serum due to the injection of the antigenous substance which in
is instance is known as the precipitinogen, this precipitin, which is
definite antibody, may be used for the immunization of another
imal and an *antiprecipitin* produced; that is, a body which will
mbine with the precipitin in such a way as to prevent precipitation
hen this substance is combined with the precipitinogen. This
then, in fact, an *antiantibody* and is practically the only example we
ve in immune reactions of such a substance. The antiantibody is
e limit for antibody formation.

The Precipitinogen.—As before stated, the precipitinogen is any
otein substance which will cause the formation of precipitins. Cer-
n of the precipitinogens are composed of two groups, one which is
ermostable and another which is thermolabile. Therefore, when
ese precipitinogens are heated and this thermolabile substance de-
oyed there results a substance which is exactly similar to the precipi-
d produced by heating the precipitin. Such bodies are known as

the *precipitoids of the precipitinogen* in distinction from the *precipitoic of the precipitin.* These precipitoids retain their power to combine wit precipitin, but no precipitate results on such combination.

The Precipitate.—When precipitin and precipitinogen combine requires some little time before precipitation occurs. This is dependei upon the temperature (37° best) and certain other factors. Tl presence of the trace of organic acids materially facilitates this reactio Furthermore, the reaction will not take place without the presence certain electrolytes or salts.

Coprecipitins.—The phenomena of "group precipitation" does n occur as often as does "group agglutination." The bacterial precipiti are very markedly specific but some of the blood precipitins are n so specific. For example, in a case where two rabbits have been ir munized, one with the blood serum of man and the other with the bloc serum of the monkey, it is found that the serum of the rabbit immuniz to human blood serum will precipitate monkey blood serum to a le degree, of course, than human serum. This is due to the fact that the are certain chemical substances in common in the blood sera of tl monkey and man. There are other rare instances of coprecipiti which will not be discussed.

The Forensic Use of Precipitins.—The precipitins are of use on a count of their great specificity in the identification of various albur nous substances. They have been used, for example, in the identific tion of bloods. Before the knowledge of the precipitins was availabl the only means of determining one blood from another was by means the microscopic examination of the corpuscles. If the corpuscles we in a good condition, it was possible, for example, to differentiate betwe a mammalian and fowl blood, on account of the nucleation of the cc puscles of the latter. By the use of the spectroscope it was also possib to determine whether a particular stain was blood or not. When came to determining the exact species from which the blood came was impossible. By means of the precipitins this can be done. F example, a stain which is supposed to be blood is carefully dissolv out in 0.85 per cent sodium chloride solution and placed in a ster test-tube. A series of animals, such as rabbits, have been immuniz to the various known blood sera and after immunization their sera a drawn off. These sera contain the precipitins for the various sera a corpuscles used in immunization. These precipitins are combin

parately in small test-tubes with the salt solution preparation of the ood in question. A precipitate occurs when the corresponding pre- pitating serum is added. It is necessary, of course, to place these eparations in the incubator at 37°. By this method all types of ammalian blood may be separated from each other with the possible ception, as before stated, of monkey and human blood. In this stance it is necessary to make careful comparisons in order to deter- ine the concentration of the precipitins. The precipitins may also e used in the identification of various meats and other albuminous bstances such as eggs.

In some ways the precipitins resemble colloids and it has been shown at organic colloidal substances such as ferric hydroxide, etc., when in queous solution, may be precipitated by the addition of certain elec- olytic salts. The precipitation occurs in this instance in a very milar manner to that of the organic precipitins.

The Theories of Immunity

Various theories have been proposed which attempt to account for e resistance naturally present in animals, and the resistance which ay be artificially produced. One of the first theories proposed was e so-called *noxious retention theory* which held the view that in natural mmunity there were natural noxious substances present in the body hich prevented the growth of the infectious microörganisms. In quired immunity it was supposed that, as the result of an infection, ecific noxious substances were produced and consequently new infect- g microörganisms of the same species as those producing the original fection were unable to grow. This theory has long been discarded. nother theory, for a time prominent, was known as the *exhaustion eory*. It was conceived that natural immunity was due to the fact at the body tissues did not possess the necessary food products for e invading microörganisms and that in acquired immunity these ecessary food products were exhausted completely so that when a sec- nd infection was attempted none could possibly occur. This theory as also been discarded.

One of the most prominent theories is the one which has been eld more recently, with some modifications, namely, the *chemical de-chain theory* of Ehrlich. It is claimed that tissue cells are made

up of definite chemical substances which possess chemical side-chai which are open for chemical combination with other substance It is by means of these chemical side-chains that food products a absorbed and assimilated by the cells. Furthermore, it is by mea of these chemical side-chains that toxins and various poisons a absorbed by the cells. It seems to have been clearly demonstrate that as a result of the absorption by certain cells of the body of tox substances, particularly bacterial toxins, that the cells are stimulate and produce or open up an excess of these chemical side-chains f combination with various substances. It is conceived that if enoug toxin (not enough to kill the cells), is assimilated by the cells t chemical side-chains which are definite chemical substances will b split off from the original cell compound and escape into the circulatio It is these escaped chemical side-chains which constitute the antitoxi or bactericidal substances. In the case of antitoxins, they posse a maximum affinity for the toxin and will combine with the toxin muc more readily than the toxin will combine with the remaining chemic side-chains of the original cell compound. In the case of bacter cidal substances they will combine with the bacteria and destroy the and liberate in this way the endotoxins which may subsequently combi with antiendotoxin (?) or tissue cells. Inasmuch as no antiend toxins are ever produced, the presence of bactericidal substances i a large percent of instances is a detrimental factor. The productio of antiendotoxins by some method or other is extremely desirabl Since the majority of our diseases are due to bacteria-producing end toxins, such a product would be of immense value in combating the infections. The chemical theory of Ehrlich explains many featur of the phenomena of immunity. This theory has been the basis nearly all of the preceding discussions on the various antibodie

Metschnikoff suggested what may be called a *phagocytic theo* of immunity. According to his ideas and those belonging to h school, the phagocytes, and principally the mononuclear and poly morphonuclear leucocytes, are concerned in immunity. He explai natural immunity to toxins on the basis of an increased toxin-absor tive power on the part of these cells for toxins. He explains natur antibacterial immunity to an increased power of phagocytosis for t invading microörganism by the leucocytes. He conceived that i acquired immunity to toxins these cells develop as the result of an i

ection or artificial injection of microörganisms, an increased power of absorption of toxin and the power of producing antitoxin, and that acquired immunity to bacteria-producing endotoxins is due to the increased power of the phagocytes to ingest and digest invading microorganisms.

We find the best explanation for the phenomena of immunity in both the theories of Ehrlich and Metschnikoff. Undoubtedly certain forms or types of immunity are due to definite chemical substances known as antitoxins or bactericidal substances, while other types are due to the activity of the phagocytes.

CHAPTER III

MICROBIAL DISEASES OF MAN AND DOMESTIC ANIMALS

Diseases Caused by Molds* and Yeasts

The diseases produced by fungi in higher animals are mostly localized infections of the skin (dermatomycoses), of the mouth and throat (thrush), and of the lungs and air passages (pneumomycoses). In insects, one large series of forms, the *Laboulbeniales*, produce local affections only; many other forms from widely different groups are destructive to particular insects.

Pneumomycosis†

Aspergillosis.—The fungus disease of the lungs and air cells of birds is quite uniformly attributed to *Aspergillus fumigatus* which is widely distributed upon feed and grains as well. The agency of this species in causing disease is well established. It grows best at blood-heat. Inoculation experiments have reproduced the disease. Isolated cases are recorded where the same organism is regarded as the cause of disease in horses or cattle and even man, but not proved. Other species of *Aspergillus*, *A. flavus*, *A. nidulans*, *A. niger*, have been listed among pathogenic forms from their presence at times in diseased tissue. Whether these species are ever a primary cause of disease is doubtful.

Secondary Infections.—Spores of any species of fungus found in the locality may find lodgment in wounds, orifices open to the outside, such as the external ear or the air passages. Many of these spores will germinate in such situations. If favored by dirt, pus, mucus, or existing pathological condition the resulting growth in some species develops into a secondary infection; most species lack entirely the power to produce disease. The appearance of molds, especially species of

* Arranged generically as far as possible.

† Prepared by Charles Thom.

Mucor, *Penicillium*, and *Aspergillus*, in such situations has been frequently reported in literature. In very large measure at least such presence may be regarded as evidence of lack of care, cleanliness, of even ordinary precautions when the infection involves man.

Thrush*

The parasite of thrush, *Oidium albicans* Robin, (*Saccharomyces albicans*, Reiss), in culture produces a scanty mycelium, submerged in the

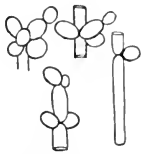


Fig. 150.—*Oidium albicans*, from a culture obtained from Kral.

substratum, which branches monopodially. The tendency to budding and to the entire suppression of the mycelium leads some to regard this form as a yeast (Fig. 150). It attacks the mucous membrane of the

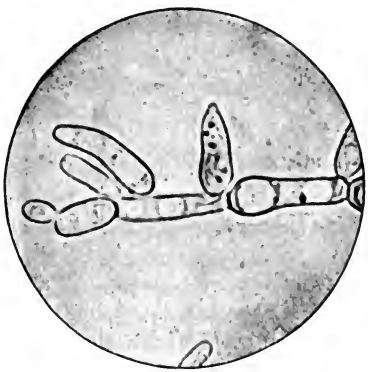


Fig. 151.—*Oidium albicans*. (*Kohle and Wassermann.*)

mouth and throat in young animals only, producing vesicles, then white membranous patches composed of the mycelium of the fungus (Fig. 151). It is to be recognized in such cases by microscopical examination. The same disease affects children and is found in fowls, calves, and colts.

* Prepared by Charles Thom.

Dermatomycoses*

The molds which cause skin diseases form a small group, with relationships to the commoner forms of fungi very ill-defined. They produce a vegetative mycelium within the tissues of the host with fertile branches which bear conidia but indicate little as to their group relationships among fungi. Certain of these diseases have been carefully studied, mostly from the pathological side.

Barber's Itch, Ringworm, Herpes Tonsurans, Trichomycosis. —The disease due to *Trichophyton tonsurans* (Fig. 152), Malm, has received many names in different languages. It attacks man and domestic animals, the ox, horse, dog, cat, sheep, hog, probably other animals as well. It is characterized by the formation of circular patches from which eventually the hairs fall. These patches enlarge radially and fuse into large areas covered with crusts with more or less discharge in the center. The fungus is recognized microscopically by examination of hairs pulled from the growing edge of the infection. The hyphæ penetrate the layers of the skin and especially surround the roots of the hairs which, when first affected, stand stiff and straight.

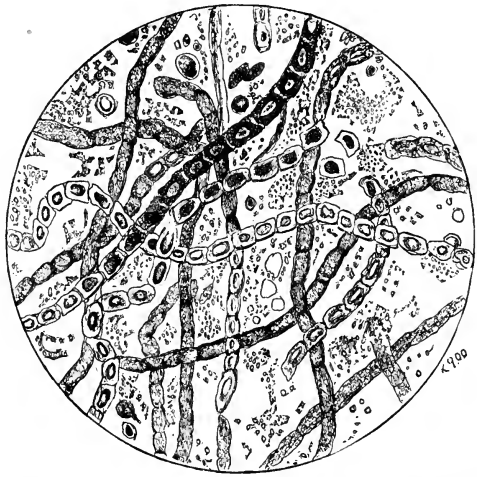


Fig. 152.—*Trichophyton tonsurans.* (*After Hyde, from Adami and Nicholls.*)

* Prepared by Charles Thom.

The appearances of the disease differ in the various species of infected animals, as also does the length of time it continues. The disease does not affect the general health greatly, since it primarily attacks the drier and more horn-like portions of the skin, but becomes conspicuous by the falling of the hair and by the scabs or crusts with accompanying itching and discomfort. Other species of the same genus have been described which produce infected areas differing in detail but similar in their general characters.

FAVUS.—Favus is caused by *Achorion schönleinii*, Remak, and affects man, cats, dogs, mice, rabbits, and fowls, and many wild animals. This is characterized by crusts, thickened at the edges and somewhat cup-shaped in center, composed of the mycelium of the mold cemented together into masses by glairy substance. Below, these crusts are in contact with the true skin. The fungus penetrates especially into the hair-follicles and hairs themselves, which later are shed. It attacks different species of animals with varying symptoms, but produces more serious lesions than those of *Trichophyton*. Favus is especially serious when it attacks man. Efforts to show that this fungus is merely a parasitic form of some species of higher fungi have failed. The diseased conditions have become so well defined and are reproduced so uniformly as to indicate a fixed habit in the organisms, whatever its source or relationship.

ACTINOMYCOSIS*

Actinomyces bovis†

This is a rather common disease of domestic animals, especially cattle. It prevails in Europe, North and South America, and is known by various names as lumpy jaw and wooden tongue. Cattle are most commonly affected, but humans, hogs, horses, sheep, and dogs are susceptible. *Actinomyces* produces a local disease which never spreads widely or rapidly.

Actinomycosis is to be considered as an infectious disease which spreads by inoculation.

The disease produced by this microörganism usually runs a chronic course and is distinguished especially by enlargement of affected parts,

* Prepared by M. H. Reynolds.

† *Actinomyces bovis* has been classified by Frost (page 108) as a species of bacteria, but, because of many features, it is here inserted with the organisms strictly belonging to molds and yeasts.—*Ed.*

by hardening of the tongue, and by suppuration. The latter is one the most constant and conspicuous characteristics. Head parts, i cluding the facial bones, are commonly affected; lungs and various oth internal organs and even the vertebræ may be involved.

The extent of injury done by this fungus depends on the locatic and size of the involved area. Usually the most conspicuous injury impaired nutrition.

There is probably but little risk to human health from actinomycos in cattle as parts of the carcass most commonly affected are not eat

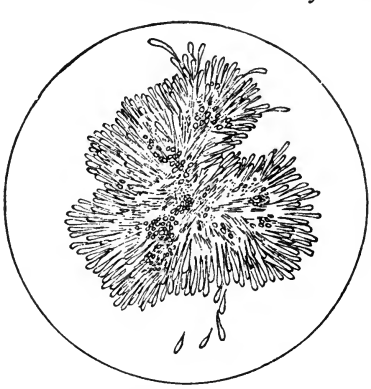


FIG. 153.— *Actinomyces bovis*. The ray-fungus from cow. (Diagrammatic (*After Williams.*)

and edible parts are usually cooked. It is generally considered th sound portions of carcasses which do not show generalized disease a fit for human food purposes.

There are apparently several varieties of *Actinomyces* all of whic are recognized for the present as *Actinomyces bovis*.

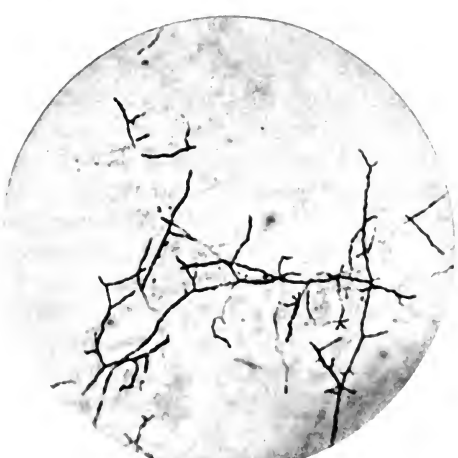
The varieties of *Actinomyces* are to be regarded as members of a ve complicated group of microörganisms higher than bacteria and a generally spoken of as fungi. *Actinomyces bovis* is commonly know as the ray-fungus (Fig. 153). Its relation to the disease of actinomycos is probably specific but it is frequently aided by pus producing bacteri

It is believed that the *Actinomyces* vegetate on various grasse especially wild barley, and that infection occurs by inoculation with th awns and barbs of such grasses through the mucous membrane of th mouth or other portions of the alimentary tract.

Infection by inoculation is the most common method of introducing ıe disease; but infection by inhalation evidently occurs in some cases. : seems probable that some special stage of development for the *Acti-*
›myces is necessary either within the diseased animal body or upon ›me plants, in order that it may be able to infect animal bodies, for irect inoculation by pus has usually given negative results. Inocula-on by bits of diseased tissue occasionally gives positive results.

It is evidently not a producer of active toxins for the disease dis-ırbances are apparently due to harmful growth in the tissues and to :condary infection.

Suppuration is one of the conspicuous features as is also the develop-ıent of much new granulation tissue which tends to degenerate at the :nter. Soft organs affected by this parasite show a tendency to multi-le abscesses.



IG. 154.—Actinomycosis. *Actinomyces bovis*. Preparation from a pure culture. ×1000. (*After Williams.*)

Actinomyces bovis grows rapidly on a variety of laboratory media. On glycerin ıar the colonies develop into transparent drop-like bodies in four or five days at 3°. Old colonies become white or yellowish with a powdery surface. The cultural ıd other peculiarities vary much and according to the variety under observation. ›me varieties appear distinctly aerobic and others anaerobic. As a rule it liquefies ·latin growing in spherical masses which settle to the bottom of the liquid. Fila-ıents appear in artificial growth which are very long and slender, and about 6μ

in diameter, and show true branching (Fig. 154). The young colony is a loo mass of filaments; older colonies become dense and felted. Rod-shaped and spheric forms appear in artificial cultures, and some filaments develop conidia. Culture especially those containing the round forms, are very resistant to heat, light, dryin and disinfectants. Stains easily. Tissue section stained with carmine followed b Gram's method gives good results, the thread showing dark and clubs red. Carmi followed by Weigert gives a beautiful stain. May be recognized as visible granul found floating in the pus in case of suppuration, or embedded in tissue. The granules vary in color; some are clear or yellow; others are quite dark. The color as it appears in tissue section or pus smear consists of a rosette arrangement. Th central portion of the colony is a dense mass of mycelium and spherical bodie From this felted central mass, there extend rays or club-like bodies. Club-shap enlargements at the ends of filaments frequently appear and are regarded as distinguishing characteristic of *Actinomyces*. This organism is usually destroy at 75° for thirty minutes. Final diagnosis must rest upon actual demonstratio under the microscope which is not difficult. The granular masses may be washe in normal salt solution; and examined unstained, or stained in diluted carbol fuchsi

Escape from the diseased body is usually in pus discharged fro actinomycotic abscesses. In case of open lung or intestinal lesio it may be discharged through the trachea or intestines.

Very little is known concerning the disseminating agents exce that the sharp awns of barley and some similar bodies from oth wild grasses have been found carrying actinomycotic infection. The appears good reason for believing that such awns frequently serve t spread the disease. Actinomycotic pus scattered over fodder, manger and feed racks probably serves indirectly as a source of disseminatio

Actinomycosis is not a disease of rapid or extensive disseminatio Control work is usually confined to isolation, to proper dispositio of diseased animals and to suitable disinfection. It is recognized i sanitary legislation that very many actinomycotic carcasses are f for food purposes and should not be condemned.

Actinobacillosis.—Actinobacillosis is probably to be distinguishe from actinomycosis. It is very similar in subjects affected, in histo and clinical evidence, but apparently different as to specific caus The cause of actinobacillosis seems to be a very small bacterium foun also in rosette-like masses resembling those of *Actinomyces*.

Mycetoma (Madura Foot)*

This disease is endemic in India, especially in Madura, and is foun in other warm countries.

* Prepared by Edward Fidlar.

It is a chronic inflammatory process found most commonly in the ft, occasionally in the hand but very rarely elsewhere. It is charac-tized by swelling and irregular deformities of the part with the currence of sinuses whence there is a purulent discharge containing gnules suggesting those of actinomycosis. These granules may be vitish, yellowish, reddish, or black in color.

The causative organism is generally regarded as a fungus. It inot unlikely that some cases of the disease may be confused with ainomycosis. Several different molds have been described, some of vich have been classed as *Aspergilli*, while others have been given new mes. It is probable that, while the disease is a fairly well-marked chical entity, the etiological agent varies in different localities.

Successful inoculation of the monkey with the white variety and of geons with the black variety has been recorded.

Mycotic Lymphangitis*†

*Saccharomyces farciminosus**

The disease caused by this yeast-like fungus has been called Japa-se farcy, epizootic lymphangitis, and mycotic lymphangitis. This ease was first recognized in the United States in 1907. It has already en found in Pennsylvania, Iowa, California, and North Dakota.

Saccharomyces farciminosus produces a slow, chronic, contagious ease of horses and mules. Cattle appear susceptible but rarely ow clinical symptoms of infection.

This *Saccharomyces* involves especially the superficial lymphatic ssels and glands, but internal organs are occasionally affected. e disease is essentially local, constitutional disturbances being slight. e disease produced is fatal in about 10 to 15 per cent of cases affected t is much more serious than these figures would indicate. Other rses that do not die are rendered useless for service, the sale value ng ruined in many cases.

The lesions produced by this parasite resemble most closely the cy form of glanders but may be easily distinguished by quite different

* Prepared by M. H. Reynolds.

† Work done by Paige, Frothingham and Paige, Meyer and others raises questions concern-specific etiology and proper classification, but it is deemed wise to continue this recognition classification for the present.

ulcers. The pus is thick, creamy, and usually yellow, whereas the from the farcy buds is clear and viscid. Farcy cases respond to mallein test; lymphangitis cases do not.

It seems to have been well established that *Saccharomyces fa minosus* is the direct cause of mycotic lymphangitis—at least of form of it.

The *Saccharomyces* grows in the animal tissues and by its prese and products acts as a direct exciting cause of the disease. Entra is effected through inoculation wounds which may be very su ficial and very trivial, most frequently perhaps on the legs, should and neck. The incubation period varies from a few weeks to sev months.

This *Saccharomyces* is distributed through lymph vessels, chi superficial ones, the nodules appearing first near the point inoculation.

The tissue changes produced are infection, inflammation, suppuration of the lymph vessels and glands. At first the lym vessels enlarge and harden; then nodules develop under the skin al the course of the vessels. These nodules suppurate and the sm abscess cavity fills up with bright red granulation tissue. The en limb may enlarge very greatly by reason of excessive connecti tissue formation, and the greatly thickened skin.

Saccharomyces farciminosus is a yeast-like fungus, ovoid in shape and 3μ t long by 2.5μ to 3.5μ broad. This fungus grows slowly under artificial condition agar and bouillon after inoculation with pus from an abscess. It reproduces budding and does not stain well by common laboratory stains. Claudius' met of staining gives good results.

Cases should be isolated and stables disinfected by the free us very strong disinfectants as this *Saccharomyces* is not easily killed ordinary disinfecting solutions.

Another mycotic organism has more recently been report in the United States as causing a lymphangitis very similar clinic to the lymphangitis caused by *Saccharomyces farciminosus.* C supposed to have been plain cases of the *Saccharomyces* form sho on laboratory examination a *Sporothrix* acting as the direct ca These workers* reproduced cases by inoculation and recovered

* Sporothrix and Epizootic Lymphangitis, Paige, Frothingham, and Paige. Journ Medical Research, Vol. XXIII, No. 1. This has been previously reported by Shenck, toen, and others for the human.

ganism differing very materially from *Saccharomyces farciminosus.* e case history and lesions produced parallel very closely those pro-ced by the *Saccharomyces.* This *Sporothrix* seems to have great ality, remaining virulent in dried pus at a temperature of $-7°$ for ee months or more.

The same organism has been recovered from similar lesions of the man where it was apparently acting as the direct exciting cause. this be confirmed, we have two very different organisms capable producing a similar mycotic lymphangitis.

DISEASES CAUSED BY BACTERIA*

Botryomycosis†

Botryomyces equi

We have typically in this disease closed abscesses with very tough rous walls and slow development. These abscesses involve espe-lly subcutaneous and intermuscular connective tissue, although ical lesions have been found in various internal organs.

This affection usually appears in horses, but botryomycosis has been nd in cattle and swine.

The identity and proper classification of a specific microörganism still in dispute. Johné found *M. ascoformans* acting as an etiolog-l factor. Kitt and others found micrococci which could not be dis-guished from *M. pyogenes.* Moore found a variety of pyogenic rococci and streptococci apparently serving as causative agents al reports one case of an enlarged spermatic cord where he found a gus resembling *Actinomyces bovis.* In his later work Moore appears identify the botryococcus of Bollinger with *Micrococcus pyogenes.* hers identify *Botryomyces equi* with *Staphylococcus pyogenes aureus,* e.

Primary infection occurs by inoculation and not infrequently lows surgical operations, *e.g.*, castration. The primary infection y then lead to involvement of internal organs by metastasis. The

* Arranged alphabetically under each of the following families: *Coccaceæ* (*Micrococcus, Streptococcus*), *Bacteriaceæ* (*Bacterium, Bacillus, Pseudomonas*), *Spirillaceæ* (*Microspira*).

† Prepared by M. H. Reynolds.

local effect here is that of an irritant and both irritant and tissue response appear to resemble those that occur in actinomycosis.

Botryomycosis is easily distinguished from actinomycosis by microscopic examination. Cases that resemble the farcy form of glanders are easily distinguished by mallein test, by laboratory animal inoculation and by lack of adjacent lymph-gland involvement.

Gonorrhœa*

Micrococcus gonorrhœæ

Gonorrhœa is one of the most prevalent of the bacterial diseases and is found throughout the civilized world and is confined to the human race.

The urogenital tract is the most frequent seat of infection but orchitis, severe conjunctivitis, arthritis and endocarditis are not uncommon and a septicæmic condition may also occur. Ophthalmia neonatorum is due to this organism. The ordinary infections of the urogenital tract have an incubation period of from two to eight days. The inflamed mucous membranes give rise to more or less pain and yield a thick yellow discharge.

While the fatality due directly to *Gonococcus* infection is not high, the frequent tendency to chronicity renders it one of the most important diseases.

Gonorrhœa has been known from the very earliest times. In 1879 the diplococcus was pointed out by Neisser as the probable cause. Bumm in 1885 first cultivated it on coagulated human placental serum.

The microörganisms can be easily stained in the typical early discharges where it occurs in pairs and for the most part within cells (Fig. 155).

For isolation, agar media should contain human blood or blood serum or ascitic fluid, though the swine-serum-nutrose medium of Wassermann is also good. The fluid must be sterile and must be added to melted nutrient agar at about 45°. The *Gonococcus* is about 0.6μ to 0.8μ in diameter. It is usually seen in pairs; where the adjacent sides of the cocci are flattened the long diameter of the pair reaches as much as 1.6μ; non-motile and forms neither spores nor capsules. It stains readily with the aniline dyes and is Gram-negative. The temperature range is 30° to 38.5° with an optimum of 37.5°. Aerobic conditions are preferred though a slight growth may

* Prepared by Edward Fidlar.

obtained anaerobically. The most favorable reaction of artificial media is said to about 0.6 per cent acid to phenolphthalein. On serum agar or similar suitable edia, colonies appear in twenty-four hours as fine slightly elevated, translucent opalescent spots frequently referred to as "dew-drop" colonies. They possess faint bluish or grayish white color with a slightly marked concentric or radial riation with a scalloped margin and finely granular center. In serum broth there ay occasionally be a uniform clouding though, as a rule, there is a finely granular diment somewhat slimy with clear fluid above. Only in exceptional cases has owth been observed in gelatin because of the unfavorable temperature. On

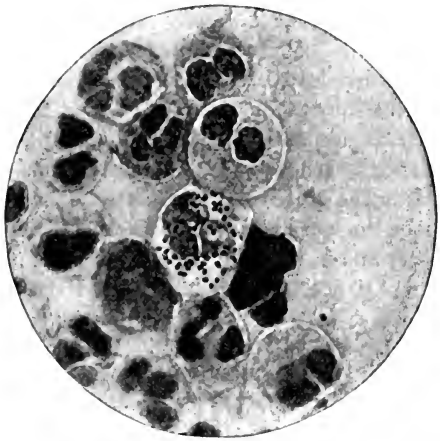


FIG. 155.—*Gonococci* and pus cells. × 1000. (*After Williams.*)

pissated blood serum growth may sometimes be observed as discrete pale yellow- or brownish colonies. Dextrose is changed with the development of acid but gas. Alkali is not formed in any medium by typical strains. No gas, indol pigment are formed. The toxins are intracellular and quite thermostable. sistance is very slight toward external influences. Cultures undergo rapid tolytic changes and die out at room temperature, often within forty-eight hours. temperature of 41° to 45° will kill in a few hours. To light and drying they are o very sensitive, and are rapidly killed by the ordinary disinfectants.

Animals inoculated subcutaneously or intraperitoneally show symptoms of poisoning with suppuration and necrosis locally and may succumb.

The virulence of the organism is variable. They may apparently lie rmant or at least they are very slightly active in chronic conditions one individual but set up an acute gonorrhœa when transferred a second person.

The organism gains entrance to the urethral mucosa or conjunctiv usually by direct contact and it is doubtful if the disease could b carried by any infected article later than twenty-four hours.

The organism is found at the local lesion and has been obtaine from the fluid of affected joints, and from the blood in the septicæm cases.

A general immunity is seldom if ever developed in man following a attack of gonorrhœa. There has been demonstrated in the blood serun however, a complement-fixing antibody which, while not absolutel constant, is present with sufficient frequency to be an aid in diagnosi

Injections of cultures into animals give rise to agglutinins, bacter cidal and complement-binding bodies.

The diplococcus is eliminated only in the purulent discharge.

Great importance attaches to the fact that persons may harbor th *Gonococcus* long after the acute condition has disappeared and when th coccus seems to be no longer harmful to its host. Such cases brin about untold misery and form one of the most difficult problems in bot medical and social science. It has been stated that *Gonococci* hav been found as late as twenty years after the primary infection.

The most extended and successful prophylactic measures have bee carried out in the armies and navies of various countries by the use germicidal solutions whenever there has been any chance of exposu to infection. The use of germicidal solutions in the eyes of new born infants is practically universal as a preventive measure again ophthalmia.

Epidemic Cerebro-spinal Meningitis*

Micrococcus meningitidis

Cerebro-spinal meningitis may be caused by different bacteria su as the pneumococcus, streptococcus, staphylococcus, influenza bacillu tubercle bacillus, etc., but the greater proportion of cases of acu meningitis, those of the epidemic type, are due to the meningococc or diplococcus intracellularis meningitidis.

Epidemic meningitis has been described chiefly in Europe and Ame ica and appears to have been first clearly defined in 1805. Whi sporadic cases occur, the disease usually exists in the epidemic for

* Prepared by Edward Fidlar.

eginning in the fall, continuing during the winter, and declining in the pring. Of late years it would seem to be on the increase.

The incubation period is unknown.

There is considerable variety in the character of the cases. As a ule the invasion is sudden with headache and vomiting as prominent ymptoms. The headache usually increases, with disturbances of ision, restlessness, and pains and rigidity in the muscles of the back nd neck. The temperature is irregular and variable, the usual being bout 101° to 102°. Herpes occurs frequently and a purpuric rash is ommon, especially in the severe cases, so that the term "spotted fever" as sometimes been given to the disease. The patient usually passes nto a stuporous state, though delirium may occur before it. Death nay occur in a few hours (fulminant type) or within a week, or occa- ionally may be postponed as late as six months. In all favorable ases the convalescence is slow.

A fibrinous exudate which occurs chiefly at the base of the brain nd the presence of pus cells in the cerebro-spinal fluid are prominent athological features, but in the fulminant cases the gross pathological ndings may be surprisingly insignificant.

The mortality of the disease before the advent of serum treatment vas about 70 per cent of the number of cases, but this has been reduced o about 30 per cent according to Flexner's figures.

The demonstration of the organism in the cerebro-spinal fluid of ne typical case may sometimes be an easy matter, but at other times nay require a prolonged search. It appears as a Gram-negative coccus, ngle and in pairs, frequently within pus cells but occasionally extra- ellular. From the usual case it can be obtained in pure culture by owing the sediment from the spinal fluid upon suitable media. The mount of material planted should be abundant and to supplement nese first cultures it is well to incubate the fluid at 37° for twelve to ighteen hours and then make further inoculations. The media used re usually fresh blood agar, serum agar and occasionally Loeffler's lood serum.

As found in culture media the meningococcus will show swollen nvolution forms often in comparatively young cultures. There are no pores, flagella nor capsules. It can be stained readily by the aniline yes and with methylene blue will sometimes show metachromatism. t is Gram-negative.

The temperature relations are of some importance in identifyin the coccus. It has a minimum temperature of about 25°, an opt mum of 37° and a maximum of 42°. Its atmospheric requirements ar those of an aerobe.

Upon suitably enriched agar media the colonies are small, grayis and glistening with a smooth outline and granular center. In brot growth is slow and occurs at the surface. Only rarely is growth ob tained on gelatin media chiefly because of the unfavorable temperatur required. There is no change in litmus milk. Acid is formed fro dextrose and maltose.

The toxins of the meningococcus are probably intracellular.

The resistance of the organism to unfavorable conditions is ver slight, and it undergoes autolytic changes almost with the same rapidit as does the gonococcus.

Meningitis due to this coccus does not occur naturally in animal but it has been produced in monkeys artificially. Laboratory anima inoculated subcutaneously, intraperitoneally or intravenously with sufficiently large dose will die without developing meningitis.

Animals immunized by graded doses show specific agglutinin opsonins and lysins. Horses so treated yield a serum which in som hands has given very favorable results. In recent epidemics in th British and Canadian armies, however, the mortality has ranged fro about 40 to 50 per cent and the serum has been distinctly disappointing

As the germs leave the body in the discharges of the nose and mouth the prevention and control of the disease would appear at first though to be an easy matter, but the occurrence of carriers and ignorance o the factors which govern the virulence of the infective agent and th individual's susceptibility make epidemic meningitis a very difficu problem from the standpoint of public health.

Infectious Mastitis*

Infectious mastitis or mammitis (inflammation of the udde appears in isolated outbreaks and is serious for the individual owner an individual herd, but it never spreads widely. It may affect a larg portion of the herd and cause heavy financial losses. Infectious mast tis may have serious significance for children and others consumin

* Prepared by M. H. Reynolds.

ilk; but there is little information on this point, based on careful ork.

This is to be considered as an infectious, enzoötic disease and prob- ly not specific. There is good reason to suppose that different out- eaks have been due to several different pyogenic or pus-producing ganisms.

We cannot consider any one species of bacteria as the specific cause. arious micrococci, streptococci, and staphylococci have been found ting as causal agents.

Recent evidence indicates that udders of apparently healthy cows ay contain a variety of bacteria and that the infections may remain ore or less permanent. This is in part the explanation of recurrent ses of mastitis.

In the animal body this infection is practically limited to the udder. s discharge is either through the teat or rarely by external rupture abscess. Transmission from cow to cow is indirect, and frequently milkers' hands.

Entrance is usually effected by way of the milk ducts; thence into e milk cistern and to more remote parts of the gland. The infection ay also come by way of the blood or lymph channels to the glands. given case may thus be due to bacteria previously in the udder, the tack being determined by an area of lessened tissue resistance pro- ced by injury.

In one class of cases, the gland structures are first involved; in other ses the connective tissue frame-work is first involved. In one type of is disease caused by streptococci these microörganisms attack espe- lly the mucous membrane lining milk ducts and produce a catarrhal sease of that membrane. This is indicated by a cord-like swelling ich extends along the milk canal through the teat to the milk cistern. is infection frequently leads to "blind quarter;" *i.e.*, to closure of the at canal and loss of the quarter; or this infection may lead to the for- ation of one or more pea-like nodules along the teat canal and conse- ent obstruction.

In many cases the lactose is decomposed by the invading organisms, ding to the formation of organic acids. These acids produce coagu- ion. The coagula soon obstruct the milk ducts and alveoli and the creting cells degenerate. The invaded tissues may suppurate or even come gangrenous.

These infections are indicated by dullness, lack of appetite, feve inflammation of the udder, and by small nodules or cord-like swellir within and lengthwise of the teat.

It must be borne in mind that the infecting microörganism is tl thing to be controlled. Outbreaks of this disease frequently have orig in infected cows added to the herd. Some cows are unsuspected "ca riers." New cows should be suspected until found free by caref examination.

Affected cows should be isolated if possible, and always milked las Their milk should be boiled and fed to hogs, and the milkers' hai suitably disinfected.

Malta Fever*

Micrococcus melitensis

This disease is endemic along the shores of the Mediterranean, South Africa, India, China, the Philippines, and the West Indies.

The period of incubation is usually about six to ten days.

The ordinary variety shows an intermittent or undulatory fev which may be protracted to six months or more, accompanied by const pation and general debility with various complications such as neura gias, arthritis, orchitis, etc. Relapses occur after periods of absence symptoms. Malignant cases are described which may be fatal in week or ten days. The mortality is 2 per cent and no characterist pathological changes are found.

The etoliogical factor is *M. melitensis* and was described by Bru in 1887.

The organism can be obtained from the blood and in many cas from the urine. The most recently reported favorable medium f blood cultures is peptone broth with the addition of bile.

It is generally recognized as an oval coccus, although it is also described as bacillus. Its maximum measurements have been found to be 0.8μ by 0.53μ, i minimum diameters 0.55μ by 0.4μ. It occurs singly, in pairs, in irregular groups a in short chains. (Recently the organism has been described as motile and possessi a single flagellum at the extremity of the long diameter of the oval coccus.) stains by ordinary aniline dyes and is Gram-negative. It grows slowly at roo temperature, better at body temperature and does not seem to be markedly sensiti to acidity or alkalinity of reaction. It grows aerobically. On plain agar aft

* Prepared by Edward Fidlar.

bout forty-eight hours small whitish to yellowish colonies appear. Growth has een observed in broth in eighteen to twenty-four hours, on gelatin in eight or nine ays, and the latter is not liquefied. It has been found to grow on acid potato and n acid or alkaline urine.

Human beings and animals eliminate the organisms in the urine, and he milk of goats has been found to be a prolific source of infection. With proper regulations in regard to goats' milk the disease has been reatly reduced.

Staphylococcic Infections*

Boils, Abscesses, Wounds, Osteomyelitis, Pyemia, Etc.
Micrococcus pyogenes var. aureus, etc.

Infections of this order are found throughout the world and because f the association of staphylococci and streptococci with the large najority of purulent inflammations, these organisms are called the yogenic cocci.

No specific disease is produced, but chiefly boils, circumscribed bscesses, infected wounds, osteomyelitis, pyæmia, etc. The symptoms lone will not indicate whether staphylococci or streptococci are resent, but a low grade of infection with more pus and less constitutional disturbance tends to indicate the former, and staphylococci tend o pyæmia rather than to septicæmia.

Pasteur, Koch, Ogston and Rosenbach established the importance f these organisms.

Staphylococci in pus stain readily with aniline dyes. Pure cultures an be obtained by plating or streaking on plain nutrient agar.

While several different forms are found in pathological conditions, he *M. pyogenes var. aureus* is by far the most frequent, and it is described here as a type.

M. pyogenes var. aureus is a spherical coccus about 0.7μ to 0.9μ in diameter though orms 0.4μ to 1.2μ have been noted. On solid media the organism may be found olitary, in pairs, or in rows of three or four, but characteristically in irregular groups ke bunches of grapes. In liquid media the single and paired arrangement is most requent. No spores, no capsules and no flagella are found; the organism shows narked Brownian movement, like other cocci; Gram-positive. The temperature ange of growth is from about 10° to 43° with an optimum about 30°. Aerobe and

* Prepared by Edward Fidlar.

facultative anaerobe. It grows readily on all routine media, preferring a reactic slightly alkaline to litmus. Growth on plain agar is rapid and abundant. Aft twenty-four hours there appear round grayish-white or yellowish colonies about mm. in diameter, smooth and raised above the surface of the medium. Micr scopically, the colonies are regular in outline and finely granular. The cha acteristic orange-yellow pigment may not appear until later or if already present twenty-four hours, deepens with further growth. In broth, growth is also rap and causes a diffuse clouding with a thin pellicle and a heavy sediment after sever days. In gelatin, colonies are as on agar and sink into cup-like depressions as t medium is liquefied. Liquefaction is rapid with some strains and slower with other and in old cultures is of a funnel or saccate type. It is due to a thermolabile fermen like substance known as gelatinase. In milk, the staphylococcus grows readil and causes coagulation sometimes early but usually in three or four days' tim On potato growth is usually abundant; it is not as moist nor as smooth as on ag and is slower. Pigment is developed usually to the highest degree and sometim cultures appearing white on agar develop pigment on potato. On inspissate blood serum growth is usually moist and abundant. Occasionally the growt sinks slightly into the medium suggesting partial liquefaction. In dextrose, lacto and saccharose media acid is produced, but no gas. Acid is a constant produc Formic, lactic, butyric and valerianic acids have been found and probably oth fatty acids occur. Some authorities state that indol is formed but negative resul are the rule. Nitrites are formed by the reduction of nitrates. A characterist odor from cultures is due probably to the presence of fatty acids. The pigmer appears in aerobic cultures and is absent in anaerobic cultures. It is insoluble i water but soluble in alcohol, chloroform, ether and benzol. The toxins are largel intracellular. A thermolabile, hæmolytic substance may be found in the mo virulent strains after about ten days' growth in moderately alkaline broth and ca be freed by filtration through porcelain filters. Another soluble toxic substance found, causing the death of leucocytes—*leucocidin*. It is considerably less stab than the *staphylo-hæmolysin*. The staphylococci are among the most resistant the non-spore bearing bacteria. Sometimes 60° for a full hour or even longer necessary to kill watery suspensions; 70° is usually necessary to kill in ten minute If organic material is present the resistance is, of course, much greater. Low ten peratures have little effect and it has been stated that 30 per cent have survive thirty minutes' exposure to liquid air. To direct sunlight and drying staphyloco also show considerable resistance and may be found in dried pus for several month Resistance to germicides is also somewhat greater than that of other vegetativ bacteria, and is increased especially in the presence of organic material. In water suspensions staphylococci are killed by 1 : 1000 mercuric chloride in ten to fiftee minutes, by 3 per cent carbolic acid in two to ten minutes and by 5 per cent forma dehyde in the same time.

Man seems to be considerably more susceptible to staphylococcic in fections than animals. Of the latter rabbits and mice and guinea-pig are susceptible in this order.

The virulence of the organism shows considerable variation and is usually increased by successive passages through animals of the same species while remaining unaltered for animals of other species.

Subcutaneous inoculation usually results in abscess formation. Virulent cultures injected into the peritoneal cavity of animals may kill in forty-eight hours to a week or even longer with pyæmic abscesses especially in the kidneys. Malignant or ulcerative endocarditis has been experimentally produced by intravenous injection when the heart valves have been injured, chemically or mechanically. Osteomyelitis has also been experimentally produced.

In man simple rubbing of virulent cultures into the skin is often sufficient to produce a furuncle.

Upon entering the tissue the cocci are strongly chemotactic and pus inevitably results. With virulent cultures the leucocidal substance is more or less strongly active. The organism may be limited to the first abscess or by invasion of the blood stream multiple abscesses result. In these cases, which are usually fatal, the organism will be found throughout the body.

Immunization can be secured by repeated injections of cocci dead or alive in graduated doses. The serums possess slight bactericidal and agglutinating properties, and a high degree of opsonic power. The latter property is probably the most important.

The serums of immunized animals is protective only when used slightly before or along with the injection of the organisms and is consequently of little practical value. Active immunization, however, is being extensively practised particularly with the autogenous strains. Leucocytic extracts have also been successfully though not so widely used.

The prophylaxis of staphylococcic infections is the same as for other pus-producing forms.

Several other kinds of staphylococci have been found associated with pathological conditions, the most important of which are *M. pyogenes var. albus*, *M. epidermidis albus* (Welch), and *M. pyogenes var. citreus*. The first seems to be slightly pathogenic, and rarely produces severe infection. It is distinguished from the aureus by lack of pigment.

The second variety appears to be an attenuated form of the other.

The third variety is distinguished from aureus and albus by the development of a lemon-yellow pigment.

Streptococcic Infections*

General Septicæmia, Puerperal Septicæmia, Erysipelas, Etc.
Streptococcus pyogenes

Streptococcic infections are endemic among all races and under al social conditions. In the days before antisepsis and our knowledge o the transmission of infectious diseases, erysipelas and puerperal sep ticæmia occurred in epidemics that were the scourges of surgical an lying-in hospitals.

When the work of Pasteur and Lister became fully comprehende such epidemics ceased to exist.

Natural streptococcic infections have been described in horses an cattle and among the laboratory animals, but as a rule such disease i much rarer in animals than in the human being.

The period of incubation is probably about one to three days.

The symptoms of septicæmia begin with a rapid rise of temperatur which may reach 105°F. or even higher. Chills accompany the feve and are often severe. The pulse is rapid, irregular and weak and th respiration labored. There may be vomiting and constipation o diarrhœa. Headache is more or less severe with sometimes delirium In cases lasting for several days the skin appears slightly jaundiced The urine is of the usual febrile type and, as a rule, shows the micro örganism causing the disease. Death may occur in two or three day or within a week or in milder cases may be followed by recovery.

After death from septicæmia the body tends to putrefy rapidly The glandular organs all tend to be swollen and soft, especially th spleen, and parenchymatous degenerations are found to a greater o less extent. The lining membrane of the heart and vessels is bloo stained, a rather characteristic feature of streptococcic septicæmia Bronchitis and broncho-pneumonia are usually found.

Erysipelas is an inflammation of the skin, occasionally of mucou membranes, and the name is applied now only when the condition i brought about by streptococci. The inflamed area is very definitel outlined and may present blebs of a greater or less size. Oedema ma be very marked where the skin covers loose tissue. Fever is presen with its usual accompaniments. There may be vomiting, constipatio or diarrhœa. There may be severe headaches or delirium. In fata

* Prepared by Edward Fidlar.

ases, death may occur without any apparent complication, or it may llow meningitis, pericarditis, nephritis or some other sequel. In simle uncomplicated fatal cases the liver, kidneys and spleen are swollen nd soft and show degenerative changes in the gland cells.

Pasteur, Koch, Rosenbach and Fehleisen divide the earlier honors in ne gradual working out of the relationships of streptococci to disease.

Blood culture in plain broth in the case of septicæmia or inoculation f plain nutrient agar from pus are practically always successful. rowth is never luxuriant on the ordinary media. Cultivation from ases of erysipelas is less easy because most of the organisms are found t the margin of the lesion and are difficult to reach.

In exudates a stained smear will usually demonstrate the chainrming coccus at once.

The cocci vary in size from 0.4μ to 1μ. In shape the organisms may be rounded oval or with one aspect flattened when occurring in pairs. The chains may be long short and a grouping into pairs is frequent even within the chain. There are no ue spores developed and the organism is non-motile. Capsules are not found on e majority of streptococci. Staining the organism is easily accomplished with the dinary aniline dyes. It is Gram-positive. The temperature range in which reptococci are capable of growing is about from 15° to 45°, the optimum temrature is about 37°. Streptococci are, as a rule, aerobes and facultative anaerobes rict anaerobic species are said to have been isolated from fæces. The reaction of edia should be slightly alkaline. Acid production is a striking feature of this ganism and has a decided inhibitive effect upon its growth. Concerning the tion on carbohydrates this organism typically forms acid from monosaccharides, ctose, saccharose, and salicin. Gas is never produced. Nitrates are reduced by me streptococci to nitrites. The production of hydrogen sulphide is characteristic some forms which have been grouped as *Strept. fæcalis*. No pigment is found her than the slight brownish tinge seen in some gelatin cultures. Typically tively hæmolytic. This power may be lost on cultivation. The toxic products the streptococci have been the subject of a great deal of investigation, but few finite facts have been discovered. When cultivated on plain nutrient agar the owth is visible in eighteen to twenty-four hours as small round translucent finely anular colonies, which possess an even or notched border, and a tendency to remain screte except when thickly sown. The center is thickened and the margins thinner. plain nutrient broth the majority of long-chained varieties produce at the bottom d along the sides of the tube a granular deposit, or small flocculi or large flakes, aving the remainder of the broth clear. A few long-chained varieties cloud the oth uniformly. The short-chained streptococci, as a rule, produce a cloudiness in e medium which remains for a number of days even though a finely granular posit accumulates at the bottom of the tube. On plates of plain nutrient gelatin e colony formation remains the same as that on agar. In stab cultures a finely

granular filiform growth appears which later may have a beaded appearance a sometimes a brownish color. The gelatin is not liquefied. Milk is a favoral medium for the growth of streptococci and a strong acidity and coagulation son times takes place. Growth on potato is said not to take place, but in some ca an invisible growth seems to occur. Loeffler's blood serum is also a favoral medium. Streptococci, as a rule, die out rapidly in cultures due to the accumulati of their own products. In pus, blood, sputum, etc., the organism may be fou alive after several weeks or even months at room temperature. The thermal dea point is about 54° in ten minutes. Direct sunlight kills within a few hours, a they are readily killed by many disinfectants.

Entrance of streptococci is afforded by any break in the surface the body. A local suppuration may be the result or it may be follow by a general septicæmic condition.

In erysipelas some local injury is also probably necessary as starting-point.

Following the local establishment of streptococci sufficient tox is elaborated to produce greater or less systemic disturbance. If septicæmia supervenes the poisoning becomes extreme and the orga isms are distributed throughout the body.

Immunity following recovery from natural streptococcic infecti is very slight if any, and never of a permanent sort. Septicæmi once established are generally fatal, and erysipelas can recur frequent

Bactericidal substances, opsonins, agglutinins and precipiti have been demonstrated in immune serums, which, however, sh little therapeutic success.

Streptococci are eliminated in the discharge of local infectic in sputum, etc., and are then probably more virulent. Infection contact from such sources is particularly dangerous. In anginas a streptococcic infections of the respiratory tract, the epidemiology practically the same as for diphtheria and pneumonia. Simila erysipelas is to be treated as a contagious disease.

In the prophylaxis of streptococcic diseases, greatest care must shown where chances of infection by the virulent strains are possib Isolation of erysipelas is universally practised in hospitals. Simila cases of puerperal sepsis and any local disease should be kept fr contact with other puerperæ. Streptococcic pus from all sour is to be carefully destroyed.

Streptococci seem to be always present on the exposed surfa of the body and are probably capable of giving trouble should a

ocal lowered resistance occur. The prevention of this may be accomlished by strict antiseptic treatment of wounds.

Pneumonia*

Streptococcus pneumoniæ

The occurrence of a diplococcus in the large majority of cases, specially of the lobar type of pneumonia, has caused this coccus to be egarded as practically specific and warrants the name of *Micrococcus neumoniæ, Diplococcus pneumoniæ,* or *Pneumococcus.* As occasional auses of pneumonia should be mentioned *Streptococcus pyogenes, taphylococcus pyogenes var. aureus, B. coli, Bact. diphtheriæ, Bact. nfluenzæ, B. capsulatus mucosus* (pneumobacillus), *B. typhosus* nd *Bact. tuberculosis.*

Pneumonia is world-wide in its distribution and is estimated to orm anywhere from 1 to 7 per cent of all cases studied in internal nedicine. It appears to be more frequent in regions subjected to udden changes of temperature.

The incubation period is two or three days of rather indefinite rodromata.

The onset of the disease is marked by a chill, pain in the side, and se in temperature. The respirations become frequent. The fever, s a rule, runs between 102° and 105°F. for from five to ten days and nen in favorable cases terminates by a sudden drop of temperature o normal within a few hours (crisis).

The most striking pathological findings are a marked congestion nd œdema of the lungs following which the lung becomes solid, rless and of a dark red color, the alveoli showing, microscopically, fibrinous exudate with large numbers of red blood cells, some leucocytes nd desquamated epithelium. Thereafter the lung becomes slightly ofter and is of a gray color, while microscopically the red cells degener-te and leucocytes are more frequent and more evident. The final age, resolution, is marked by the liquefaction and absorption of the ontents of the alveoli and the entrance of air.

Death occurs from toxæmia or complications such as carditis, mengitis, etc. Roughly about 10 per cent of all deaths are due to pneu-

* Prepared by Edward Fidlar.

monia and the fatalities form about 10 per cent of the total number c cases.

The *Streptococcus pneumoniæ* was described, as found in the spu tum, by C. Frankel in 1884.

A Gram-stained preparation of the sputum is sufficient to detect the diplococ but cultures are necessary for positive identification. Some medium richer tha the ordinary by the addition of blood or serum from man or animals is best, an may be inoculated from the blood and organs or from sputum and other contam nated sources by streaking or plating. Injection of sputum into white mice rabbits will often cause a fatal septicæmia in these animals and the coccus may the be obtained in pure culture from the heart's blood. It occurs as pairs of oval lanceolate cocci, with their contiguous surfaces somewhat flattened and the dist ends slightly pointed. From this type the organism may vary to spherical or sho bacillary forms. It may occur also singly or in chains of varying length usual consisting of not more than about six or eight individuals. Well developed capsul which may surround the single organism or the pairs and chains may be found exudates or in milk and serum media. There are no spores nor flagella. Th cocci stain readily with the aniline dyes and are Gram-positive. The capsule ca be demonstrated by several methods of which Welch's and Hiss' are the mo common. The temperature range is from 25° to 41°. It is both aerobic an anaerobic, and grows most readily in a medium slightly alkaline to phenolphthalei Besides serum or blood, glycerin, nutrose and dextrose are found to be favorab for its growth. On agar it grows in small, rather transparent, finely granul colonies, which are larger and more opaque when serum or ascitic fluid is presen Broth is faintly and uniformly clouded. Milk is a favorable medium for mo strains and typically is acidified and coagulated. On potato, growth may occ but is invisible. Gelatin can rarely be used at a temperature high enough to allo growth. When occasionally growth is obtained the medium is not liquefied. C blood serum, growth appears as small clear colonies and on the whole is better than agar. A number of special media are described of which one of the most valuable the inulin-serum-water medium of Hiss. It typically ferments, with the productio of acid, the majority of carbohydrates, even polysaccharides as inulin. On blo agar the typical organism produces a greenish zone in the medium about the growt but not a clear zone of hæmolysis as do most strains of streptococci. The d ferentiation from other streptococci is sometimes a matter of difficulty, and t following characters are of importance—the lanceolate shape, capsule formatio fermentation of inulin, absence of hæmolytic powers, agglutination in antipne mococcic sera, susceptibility to lysis by the action of bile salts. Acid is an im portant and characteristic product and, if allowed to accumulate, rapidly kills th organism. The toxic products appear to be closely united with the cell bodies an are only released when these are broken up. The resistance to heat is not gre and its thermal death-point is 52°. Light is germicidal if the cocci are not pr tected in thick masses of sputum. Drying is resisted rather well in sputum or t

lood of infected animals. To germicides the *Pneumococcus* is very sensitive and killed in a few minutes by the common disinfectants in their usual strength.

The pathogenic properties of the *Pneumococcus* for animals is somehat variable. Natural infection is not common. To artificial infection mice and rabbits have been found most susceptible, while guinea igs, dogs, rats and cats are more resistant, and birds are practically mmune probably because of their high body temperature. Mice and abbits succumb to subcutaneous or intraperitoneal injections of virulent cocci from cultures or in sputum with the development of a septicæmic condition, and in the latter case a peritonitis. By special methods lobar pneumonia has been produced in rabbits as has also ndocarditis.

Variations in virulence of the *Pneumococcus* are very marked. The irulence can be increased by passage through susceptible animals until n extremely small dose will kill a mouse. Cultures obtained from man ay vary considerably in their virulence for animals.

The organism gains entrance through the respiratory mucosa and s a matter of fact appears to be a common inhabitant of these regions. lowever the organism may reach the lung (the lobar distribution suggests sowing by the blood stream), it is certainly frequent to find positive blood cultures during the disease—a fact which accounts for the evelopment of such complications as meningitis, endocarditis, etc. he toxæmia probably arises from lysis of the organisms and it has been own that the autolysis of cultures in salt solution gives rise to a soluble oxic portion and an insoluble non-toxic portion.

Immunity to *Pneumococcus* infections can be shown to exist after an ttack but only for a short time.

Pneumococci may be considered as inhabiting the mucous membranes of the respiratory tract in the majority of people and acquire irulence only under some special circumstances lowering the general itality. In pneumonia and some kinds of bronchitis as above mentioned it should be remembered that sputum and mouth spray may ontain large numbers of virulent organisms.

Specific therapeutic agents such as antipneumococcic serums, vaccines of dead cultures and autolysates, as well as leucocytic extracts, have een tried and all with some promising results. The earlier failures ith serum therapy have been found to be due in part to the occurrence different strains. By arranging these strains into three groups with

corresponding antiserums more success has been obtained. Not one c these methods, however, has been sufficiently widely applied with suc cess enough to warrant general adoption.

The prophylaxis of *Pneumococcus* infections lies in general hygieni measures, in the destruction of sputa and avoidance of possible infectio by mouth spray, etc.

Anthrax*

*Bacterium anthracis**

Also called splenic fever or charbon; and in man, wool-sorter's dis ease or malignant pustule.

The disease has been known for centuries. It is thought that it wa one of the plagues of Egypt, mentioned as a murrain on beasts, and boil and blains on man and beast. The first accurate characterization c the disease was made by Chabert about 1800. Pollender in 1849 an Rayer and Davaine in 1850 reported that they had seen "filiforr bodies" in the blood of animals which had died of anthrax, and in 186 Davaine announced he had succeeded in transmitting the disease t healthy animals by inoculating them with blood from an anthrax in fected animal, and asserted that these filiform bodies or bacteria wer the cause of the disease. This result was attacked, and for ten year there was a fierce controversy over this idea, which was finally stille by the convincing experiments of Robert Koch in 1876. Koch culti vated the bacterium of anthrax from the blood, showed that the inocu lation of these cultures in susceptible animals produced anthrax, worke out the life history of the organism, and enunciated the cardinal require ments—which constitute the proof of the pathogenic nature of an organ ism, what later bacteriologists have named the rules or postulates c Koch.

Geographical Distribution.—The disease is very widespread occurring all over the world in tropic, semitropic, and temperate cli mates. Wherever stock are found in large numbers anthrax is usuall present. The disease ravages the herds and flocks in Russia, Siberia India, Argentina and parts of Hungary, France and Germany. Loca epidemics occur constantly in England, Canada and the United States In the delta of certain rivers the organism probably grows in the soi

* Prepared by F. C. Harrison.

in the deltas of the Mississippi and Bramaputra, and the disease is so common along the banks of many rivers (Vistula, Rhine, Seine, c.).

The anthrax organism is a large, non-motile rod, from 5μ to 10μ long and 1μ to 3μ broad. In cultures it frequently forms long threads or filaments (Fig. 156). ıe free ends are slightly rounded, but those in contact are quite square, and ghtly larger in diameter than the middle of the cell. Involution forms are tained by culture on potato or at temperatures of 40° to 42°. It forms oval spores thout distortion of the mother cell (Fig. 157). Free oxygen is necessary for the velopment of these bodies, and a temperature between 18° and 41°. Spore ger-nation is polar. By culture at 42° an asporogenous variety is formed. It stains

FIG. 156.—*Bact. anthracis.* Showing thread formation of colony. (*After lle and Wassermann from Stilt.*)

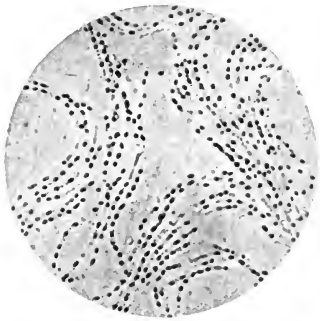


FIG. 157.—*Bact. anthracis.* Spore production. (*After Migula.*)

ıdily with the aniline dyes and also by Gram's method. Under certain conditions apsule may be seen. The organism is aerobic, in the body it grows as a faculta-e anaerobe. Its optimum temperature is 37°, minimum 12°, maximum 45°. Forms characteristic wavy and filamentous colonies on gelatin and agar, it liquefies atin, produces an arborescent growth in gelatin stab cultures, coagulates and ptonizes milk with an alkaline reaction. Thermal death-point of the spores in luids is four minutes at 100°, in hot air 140° for three hours. Mercuric chloride, 1000, destroys the spores in a few minutes, and 4 per cent carbolic acid with ydrochloric acid 2 per cent in one hour.

Zoölogically, anthrax is the most widespread of infectious diseases; ite mice, guinea-pigs, rabbits, sheep, cattle, horses and man are sceptible. Old rats are insusceptible. Von Behring, Metchnikoff

and others have shown that the serum of white rats contains a lysi capable of dissolving the bacterium *in vitro*. Pigs are occasionall infected; the carnivora generally are refractory, the bear and ca being less resistant. Most birds are insusceptible, but some sma birds, like the sparrow, are more susceptible. Cold-blooded animal are refractory.

Infection occurs: Through the food, giving rise to intestinal anthra Cattle and sheep are usually infected in this manner by spores, the ba terium being destroyed by the gastric juice. In man infection throug food rarely occurs.

Through the air. Infection by inhalation through the lungs occu in man through the medium of dust contaminated by anthrax spore hence the name "wool-sorter's disease.

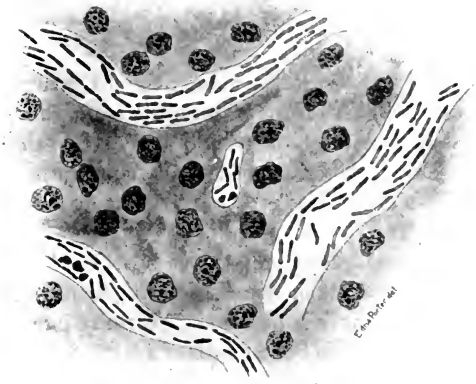
Through wounds. This method usually occurs in man and also i sheep. Cutaneous infection comes through a scratch or wound, an gives rise to a carbuncle—hence the name "malignant pustule." occurs most frequently among employees of tanneries, wool-sorter veterinary surgeons, and those whose occupation brings them int touch with infected animals, their hides or products.

The incubation period is a short one, even in the naturally occurrin disease; inoculated laboratory animals die in twenty-four to forty eight hours. The bacteria appear in the blood about fifteen hou after inoculation, and at death the blood simply swarms with the o ganism. The veins are turgid, and the blood is often very dark, an coagulates slowly. The bacteria abound in the capillaries (Fi 158). The spleen is enlarged and contains enormous numbers of th organisms. In the kidney the glomeruli and tubules are gorge with the bacteria, which pass into the urine. The bacteria can pa into the milk of females in lactation. The bacteria are also numerou in the liver, lungs and mesentery, but few are found in the muscle

Post-mortem examination of subcutaneously inoculated laborator animals shows subcutaneous œdema and enlarged spleen.

The organism is eliminated from the body in urine, fæces, mucou discharges, etc. Pastures become infected from burying anthra carcasses which have been opened or have been skinned, thus favorin the formation of spores. If buried too near the surface, the rise the ground water, or the castings of earth worms, bring spores t the surface and on to the herbage, where they may be ingested by gra

ing animals. Tanneries using anthrax-infected hides may be the cause of distributing the organism by means of effluent water which has been used for steeping hides. Many such cases have been traced in Delaware, Wisconsin and in Ontario. Hay from an infected pasture may be transported to a distant farm, and cause an outbreak of the disease. In Brazil, vultures feeding on anthrax carcasses disseminate the spores by means of their excrement, and thus spread the disease. Blood-sucking flies may also be instrumental in transferring the bacterium from one animal to another.



IG. 158.—Anthrax. The organisms of anthrax in the capillaries of the liver of a mouse. (*After Williams.*)

Season is a contributing factor. In years in which the spring floods have been very high, followed by a hot dry season, anthrax is most prevalent.

There are a few preliminary symptoms; there is usually sudden loss of appetite, trembling and convulsive movements. Often blood is seen in urine or fæces or discharged from the nose. The mucous membranes are often bluish in color, and boils or pustules may occur in various parts of the body. Death in cattle occurs in two to five days and in sheep in twenty-four to thirty-six hours. The mortality is high and intestinal cases are fatal in 80 to 90 per cent of the animals attacked.

The usual post-mortem appearances are enlargement of the spleen blood thick and tarry, bloody extravasations in the muscles and organs and bloody fluids escaping from mouth, nostrils or anus.

In anthrax-infected districts vaccination should be used. The vaccines are prepared by cultivating the bacterium at a high temperature—42° to 43°—thus forming an asporogenous race, according to methods devised by Pasteur in 1881. Two vaccines are often used, the first of very low virulence, the second more virulent. Between 1882 and 1907, 8,000,000 sheep and 1,300,000 cattle have been vaccinated in France against anthrax, with excellent results. Vaccination by toxin has been advocated by Toussiant, Hawkin, Marmier and others, but this method has not had the success of that described above.

For treatment of the disease in man, Sclavo's serum has been of considerable benefit. This serum is obtained from the sheep or ass. The animals first receive the two vaccines of Pasteur, then more virulent cultures in gradually increasing doses. A serum is then obtained which in a dose of 2 c.c. or less protects a rabbit against a lethal dose of the anthrax organism.

Animals dead of anthrax should never be opened or skinned. If doubt exists as to the nature of the disease, an ear may be cut off and sent to a laboratory for examination. Anthrax-infected carcasses may be either burned or buried at a depth of 1.8 m. (6 feet), and covered with quick-lime, and as an extra precaution the burial ground may be fenced off. The prime necessity is to prevent the formation of spores, as it has been shown experimentally that they remain in this condition for eighteen years and produce the disease when inoculated. Soiled litter, forage and the excretions of animals dead of the disease should be collected and burned.

The stalls, stables, implements and anything that has been in contact with the diseased animals should be disinfected by burning, boiling or the use of some disinfectant like 5 per cent carbolic acid.

Bacillary White Diarrhœa of Young Chicks*

Bacterium pullorum

The epidemic type of diarrhœa which is characterized in part by a whitish diarrhœal discharge, and which is now known as "bacillary

* Prepared by L. F. Rettger.

hite diarrhœa," is caused by a bacterium which belongs to the colon-yphoid group of organisms. It may be cultivated easily on the ordi-ary laboratory media, but its growth on slant agar containing Witte's eptone is delicate and bears a striking resemblance to that of Strepto-ccus pyogenes. This finely beaded growth is an important aid in ne identification of the bacterium.

The specific organism, *Bact. pullorum*, is present in the liver, lungs, idneys, spleen, heart and unabsorbed yolk of affected chicks, being ost easily obtained from the liver and yolk, when the latter is present. ome of the most common post-mortem appearances of the organs are ose of the liver and intestine, the former showing pale and congested reas, while the intestine is colorless and to a large extent void of ntents.

The disease seldom manifests itself in chicks after they have attained ne age of four or five weeks. The greatest mortality usually occurs ithin the first two weeks. The chicks become listless, and are inclined huddle together for warmth. There is loss of appetite and emacia-on. The wings droop, the back seems to shorten and the abdomen rotrudes out of proportion, causing the chicks to look stilty. The naracteristic whitish discharge from the bowel may be absent from dividual chicks, but is usually noticeable in groups of any appreciable ze.

Bacillary white diarrhœa may be transmitted to young chicks under ve days old through infected food and drinking water, as has been emonstrated repeatedly. Furthermore, chicks are often infected with *act. pullorum* before they are hatched. This is due to the fact that ne yolk of infected hens carries the specific organism in it from the time its formation in the ovary. Hence, the mother hen is the source of fection, having retained within it the bacterium in question from the me she was an infected chick, or having acquired it later in life through ntact with diseased fowls. In laying hens the infection is localized the ovary which becomes decidedly abnormal in appearance. The artly developed ova are discolored, misshapen and of all degrees of nsistency.

Ovarian infection may be determined by the macroscopic agglutina-on test which has proven itself very valuable and practicable in the rganized campaign against bacillary white diarrhœa that has been nducted in the State of Connecticut for the past two years. This

method of diagnosis has been found to be much more valuable than tl bacteriological examination of eggs.

Eradication of infected laying stock is the solution of the whi diarrhœa problem. Flocks which are at all doubtful, or which ha given a history of infection, should be tested, and the reacting fow eliminated. Better still, no eggs should be used for hatching whi have come from flocks that have shown an appreciable degree of infe tion, although reacting individuals have been removed.

Chicken Cholera*

Bacterium choleræ gallinarum

The bacterium causing this disease was first noticed by Perronci and Toussaint; later, in 1880, it was described by Pasteur, and was th first organism in which the French savant succeeded in attenuating th virulence and the first disease for which a vaccine made from atte uated organisms was prepared. Koch in 1878 described an organis of similar pathogenicity as the bacterium of rabbit septicæmia and i 1886 the term hemorrhagic septicæmia was given by Hueppe to a nun ber of infectious diseases of the lower animals in which hemorrhag spots were found in the tissues and internal organs. In 1900 Lignière discussed these bacteria, and named them as a genus, *Pasteurellose*, th specific name given depending on the animal for which it was mo pathogenic. Thus he distinguished avian, porcine, ovine, bovin equine and canine *Pasteurelloses*.

The specific characters of this group are small ovoid bacteria, often showing b polar staining when treated with the aniline dyes, non-motile, no spores, Gran negative, polymorphic, not liquefying gelatin, no visible growth on naturally ac potato, milk unchanged, no indol production, generally aerobic but also a facultativ anaerobe, virulence changeable, but usually very pronounced.

The bacterium of fowl cholera, *Bact. choleræ gallinarum*, or avian *Pasteurellose*, from 0.5μ to 1.25μ long and 0.25μ to 0.40μ broad. It develops best at 37°, and ver slowly at 20°. It loses its virulence in cultures very quickly, and it succumb readily to desiccation.

The disease is of frequent occurrence in Europe, but not often see in North America but some outbreaks have been reported in the Unite States and Canada. Unfortunately it has been confused by poultry

* Prepared by F. C. Harrison.

n with any disease characterized by excessive diarrhœa. The symp-
ns first noticed are the yellow color of droppings soiling the cloacal
thers, then diarrhœa sets in, the character of the discharge varying,
ng at times a fluid greenish mass, or a brown-red mucus, or a viscous
nsparent and frothy fluid. The bird becomes uneasy, drinks copi-
sly and with a rise in temperature to 42° to 44° the bird becomes
owsy and death follows. The period between the first noticeable
nptoms and death varies from one to three days. Chronic cases
metimes occur and in these the bacterium is found with difficulty.
e birds become infected by way of the digestive tract, from eating
d picking up material infected by the discharges of diseased birds.

Post-mortem indications are blackened combs, congestion of the
od-vessels in the organs and intestines, and punctiform or large
norrhages of the duodenum, intestines and heart. The bacteria are
merous in the blood, the pulp of all organs, and in the intestinal con-
ts. It is a true septicæmia.

If the disease breaks out in epidemic form the best and quickest
thod of getting rid of it is to kill off all the fowls, disinfect the houses,
d dig or plough up the poultry runs, and leave them two weeks before
stocking.

Chronic Bacterial Enteritis*

The disease produced by this bacterium has been demonstrated in
rmany, Belgium, Switzerland, Holland, Denmark, and perhaps
er European countries. It is known by various names, as *Johné's
ease, chronic bacterial dysentery*, and *chronic bacterial enteritis*.

This bacterium produces a chronic infectious disease of cattle in-
ving especially the intestinal mucous membrane. Other animals do
seem susceptible. The disease produced is usually fatal. Usually
most conspicuous general symptom is unthrift in spite of good
petite and good food.

This microörganism is a rod-shaped bacterium from 2μ to 3μ long
d about 0.5μ broad and is strongly acid-fast. The production of
ive toxins is to be presumed since the amount of disturbance is fre-
ently out of all proportion to the lesions found on examination
st-mortem.

* Prepared by M. H. Reynolds.

The bacteria are present in the fæces, intestinal mucosa, and sub mucosa, most frequently in the small intestines. The large intestine may be involved later.

This microörganism produces chronic, inflammatory changes of th intestinal mucous membrane, the whole intestinal wall becomin greatly thickened.

This bacterium resembles closely avian tubercle bacteria, but ma be distinguished by the fact that the avian tubercle bacterium is rathe easily grown on artificial media. This organism does not have th same pathogenic peculiarities as the avian tubercle bacterium. I seems well demonstrated that many cases of *chronic bacterial enteriti* do probably react to avian tuberculin; but this does not prove identity

So far as known the bacterium is eliminated in the manure o affected cattle and disseminated in this way. Wider dissemination i made by diseased animals moving from place to place.

The most important considerations in controlling this disease ar careful disposition of contaminated manure and isolation of suspecte animals. The manure should be used only where it can not serve t spread disease to other cattle. Sick animals should be carefully isolate and premises thoroughly disinfected.

Contagious Abortion of Domestic Animals*

Bacterium abortus

The premature discharge of the products of conception from th uterus is a not infrequent occurrence among domestic animals, an doubtless various factors may from time to time operate in its causation Injury, excessive fermentable food, or poisonous food may at time produce this result. For a long time, however, practical husbandme have recognized an epizoötic form or a contagious abortion, a definit transmissible disease, of which the loss of the fœtus is the most promi nent characteristic. This disease appears to be generally distributec in all agricultural communities. Cows, especially, are affected, but a somewhat similar if not identical disease also occurs in other domesti animals.

In 1897 Bernhard Bang discovered in the uterine exudate of a cow slaughtered during an attack of this disease before the abortion hac

* Prepared by W. J. MacNeal.

occurred, a small bacterium which he was able to grow in pure culture, and, by inoculating pure cultures of this organism, he produced the disease in cows, sheep, goats and rabbits.

The microbe is a short non-motile rod, staining with moderate ease, and decolorized by Gram's method. It does not form spores but the vegetative forms are fairly resistant to drying and may, perhaps, live for some weeks under ordinary conditions in pastures and stables. Its artificial culture requires special technic because of its peculiar oxygen requirement. The bacterium usually fails to grow in the presence of the atmospheric air or under anaerobic conditions. It requires for its development a partial pressure of oxygen somewhat less than that of the atmosphere. When inoculated into deep serum-gelatin-agar tubes and incubated in the air, the colonies develop only in a particular zone about five millimeters beneath the surface of the medium. When cultures are placed in the proper atmosphere, development on the surface may be obtained. Prolonged cultivation on artificial media obscures this peculiar property of the microbe so that old culture strains grow well under ordinary aerobic conditions.

In the diseased animal, the specific bacteria are found in the placenta and amniotic fluid, frequently also inside the fœtal intestine, sometimes in the tissues of the fœtal organs, and in the wall of the maternal uterus. The placenta appears to be the particular organ favorable to the development of the germ, and when this has been discharged from the body the abortion bacilli no longer flourish, although the infection may continue as a chronic uterine inflammation for a long time. The general health is only slightly disturbed. At the next pregnancy the disease is practically certain to reappear, and possibly again also at the succeeding one. After two or three abortions the animals appear to have acquired an immunity to the infection, and may sometimes breed normally thereafter, although some animals are permanently sterile after a few attacks of the disease.

The organisms escape from the diseased animal in the products of conception at the time of the abortion, and in the chronic uterine discharge which may continue for a long time afterward. The disease may be conveyed to other animals by contact with this material and by eating grass or other feed soiled with it. Doubtless the male is an important factor, possibly the most important factor, in transmitting the disease, although no serious inflammation is produced in him.

The control of the disease depends upon the isolation of the infected animals, cremation of infected fœtus, placenta and discharges, and thorough disinfection of the premises. Heifers and healthy cows should not be allowed to mingle with cows which have aborted, nor should they be served by a bull which has covered infected animals at any time. Local antiseptic treatment of the cow which has aborted diminishes the danger of the persisting discharge.

Contagious abortion also occurs in other domestic animals, especially in horses, sheep, goats and swine. Inoculation experiments have shown that the *Bact. abortus* of Bang can infect some of these animals. Its importance as a factor in the epizoötics of abortion occurring naturally among them is still uncertain. In horses at any rate another organism appears to be more frequently involved.

Diphtheria*

Bacterium diphtheriæ

The disease is epidemic in all large communities especially in Europe and America. It is, however, almost absent from tropical regions. Epidemics and pandemics occur in cycles. Essentially diphtheria is a disease peculiar to man. Avian diphtheria, however, is known, although seemingly due to another cause, and on rare occasions natural infection has been found in the horse.

The period of incubation is said to be two to five days.

In man the disease usually begins with lassitude and fever followed in a few hours by "sore throat." The inflamed area on the pharyngeal wall, tonsils, larynx or wherever it may be becomes in typical cases the seat of degenerative changes in the epithelium and underlying tissues with abundant fibrinous exudation resulting in the formation of a comparatively tough membrane or pseudo-membrane, which is a striking and characteristic feature of the disease. This local lesion is almost always found on mucous membranes though occasional instances of infection of wounds have been noted.

The bacterium of diphtheria was described in 1883 by Klebs in sections of typical membranes. The organisms were isolated and differentiated in 1884 by Loeffler, who was able to fulfill Koch's postulates for pathogenic microbes. Accidental infection of the human being has

* Prepared by Edward Fidlar.

ppened in the laboratory and confirmed the findings of animal inocu-
tion. The success of antitoxin treatment is further evidence of causal
lationship.

The organism is detected in the following manner: A sterile swab is rubbed gently
er the inflamed area or against any visible membrane, care being taken to touch
ner parts as little as possible. The swab is then immediately inserted into a tube of
cially prepared medium—Loeffler's inspissated blood serum—over the surface of
ich it is rubbed back and forth. The swab is returned to its own tube or left
ainst the serum and the culture and swab sent to the laboratory. After twelve to

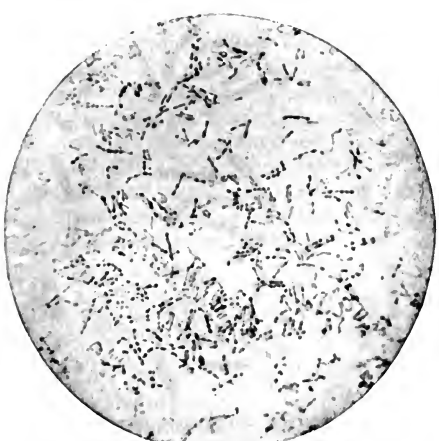


FIG. 159.—Bacterium of diphtheria. × 1000. (*After Williams.*)

eteen hours of incubation, at 37°, smears are made from the cultures and stained
h Loeffler s methylene blue. The diagnosis is made on the morphological char-
ers of the bacterium. Occurring in pure cultures the form of the bacterium is
ject to remarkable changes according to the medium and length of cultiva-
. Its size as it appears in exudates and from serum media varies from 1μ to
n length and 0.25μ to 2μ in width. The rods are straight or slightly curved,
ally not uniformly cylindrical but with swellings at the ends, or in the middle, or
gularly disposed (Fig. 159). Both ends may be rounded or both pointed, or one
nded and the other pointed. Branching forms are not infrequently found.
bacteria may appear in pairs end to end; more frequently and typically they are
ined to one another at a greater or less angle and may assume a parallel arrange-
t or the form of a short zigzag chain. The arrangement is most clearly under-
d and remembered by considering the peculiar "snapping" type of fission
cteristic of the group. There are no flagella and no spores. The cell mem-

brane is possessed of great elasticity as evidenced by the post-fission movemen
The bacteria stain readily with all the aniline dyes and retain the primary stain fai
well by Gram's method. With Loeffler's methylene blue they stain in a charact
istically irregular manner, and show metachromatic "granular" forms, "barre
and "solid-stained" forms (Fig. 160). On a basis of morphology and staini
properties, Wesbrook, Wilson and McDaniel have devised a classification which
very convenient for descriptive purposes. The minimum temperature of grow
18° to 19°, optimum 35° to 37°, maximum 40° to 41°. The bacterium grows m
readily in the presence of oxygen. Under certain conditions it will grow anae

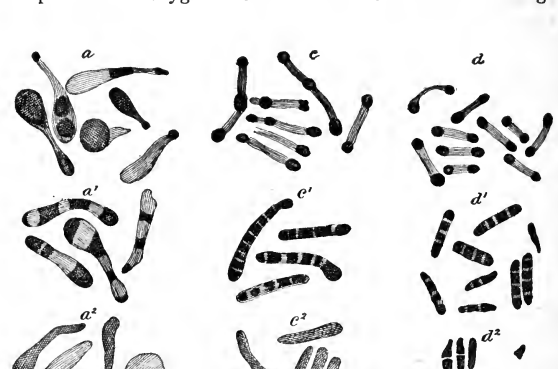


FIG. 160.—Wesbrook's types of *Bact. diphtheriæ*. *a*, *c*, *d*, granular types; *a'*, *c'*, barred types; a^2, c^2, d^2, solid types. × 1500. (*From McFarland.*)

bically. The optimum reaction of blood serum media is about +0.8. The amou
of acid which the bacterium can endure varies with the kind of acid. Gelatin is
liquefied, neither are the proteins of blood serum nor of milk. Caseinogen is
changed to casein. Some carbohydrates are broken up with the production
acid. All authorities find that the bacterium forms acid from dextrose. It
generally agreed that acid is produced from lactose, galactose and maltose. Acti
on dextrin, lactose, saccharose and glycerin is variable. The majority of workers fi
mannit is unchanged. An acid reaction in plain broth by fermentation of mus
sugar may be followed by the production of alkali. Gas is not produced under a
circumstances. No indol is formed. Most strains cause hæmolysis of red blo
cells. A true diffusible toxin is formed for the artificial production of which
broth cultures peptone, absence of sugar, an alkaline reaction and free access
oxygen are favorable factors. Growth on plain nutrient agar is not so abunda
as on Loeffler's blood serum. Colonies of two types may be found: (*a*) m
common is small grayish-white, rounded, slightly raised, almost translucent w

ore or less granular surface and dark center, the margins varying in irregularity, nd often with a thin extension spreading out from the edge; (*b*) less common, rger, more luxuriant, white, rounded, raised, granular to nearly smooth and some- hat moist. Plain broth must be slightly alkaline to litmus. About one-half of ltures grow readily and half very feebly. The characteristic growth is a finely anular deposit at bottom and along sides of the tube leaving the broth clear; a w cultures produce a diffuse cloudiness with more or less well-developed pellicle. rowth on gelatin is scanty chiefly owing to temperature at which this medium ust be kept. The gelatin is not liquefied. In milk growth takes place at com- aratively low temperature (20°) without coagulation but with acid production. n potato growth occurs especially if slightly alkaline; in the majority of cases visible; it may appear as a thin dry glaze or with a whitish or slightly yellowish nge. On Loeffler's blood serum growth is rapid. In eighteen to twenty-four urs colonies are rounded, grayish-white with a slightly yellowish tinge moderately anslucent except toward the center, smooth, moist and shiny. The margins are ly slightly irregular. With age the colonies become dull and opaque, the surface coming marked by concentric lines and sometimes also exhibiting radial striation. hermal death-points are 58° to 60° for ten minutes, 70° for five minutes, 100° r one minute. On the other hand −190° for seven days and −252° for ten urs have failed to kill in some instances. Diffuse light hinders growth. Direct nlight kills within two hours to a few days according to the medium in which the ganisms are suspended.

The organism gains entrance into the body through the mouth and ose.

The bacteria usually remain localized; they can almost always be monstrated when a definite membrane is present and often when ere is none. They are practically always found in the lung of fatal ses because of direct infection. Entrance of the bacteria into the ood stream with resulting infection of the internal organs has occurred fatal cases.

The protective apparatus concerned in diphtheria is probably dif- rent at the beginning from that involved late in the disease. Experi- entally, agglutinins, bacteriolysins and opsonins have been demon- rated in exudates and serum. While these properties may be portant in warding off an infection they appear to be of little influ- ce once the bacteria are established, and thereafter on the amount antitoxin will rest the outcome of the disease.

The toxin is strongly antigenic, the cell bodies feebly so. Aggressins ve not been demonstrated.

The organisms escape by the secretions of the mouth and nose. irect infection by coughing, sneezing and speaking probably takes

place frequently not only from the sick and convalescents but also from healthy carriers.

Control of the disease is sought by quarantine of all sick persons and the placing of restrictions if not actual quarantine on those exposed and showing the bacteria in the nose and throat.

DYSENTERY*

Bacterium dysenteriæ

Two chief types of dysentery are known, one due to a protozoan —*Amœba Entamœba tetragena;* the other due to a bacterium—*Bact. dysenteriæ.* Only the latter is here dealt with.

Acute dysentery in an epidemic form is found chiefly in Asia, sometimes in Europe and in the Philippines. In this country occasional small epidemics and certain types of summer diarrhœas of infants have been shown to be due to *Bact. dysenteriæ.* The disease occurs naturally only in man.

Dysentery is an intestinal disorder usually acute and, in its epidemic form, very severe, marked by a flux in which there is the frequent passage of blood and mucus with severe tenesmus and pain in the abdomen. The fever accompanying this may reach 104° and in the severe cases delirium and death may result. In Japanese epidemics the fatality has reached 25 per cent or more.

The pathological findings are most marked in the intestine where the mucosa is swollen and hyperæmic, with more or less hæmorrhage and extensive necrosis.

Shiga in 1898 discovered a bacterium in the stools of persons suffering from the disease and the organism was agglutinated by the blood serum of the patients. He found the same organism repeatedly in a considerable number of cases.

The results of many other investigations have demonstrated the presence of several forms conforming in general to the type described by Shiga but showing some difference in fermentation properties; these are sometimes known as para- or pseudo-dysentery bacteria.

The constant presence of the organism in the epidemic type and the fact of agglutination leave little doubt as to the etiological relation. A

* Prepared by Edward Fidlar.

riminal fed with a pure culture of Shiga's organism developed the ypical disease.

The organism can occasionally be isolated in almost pure culture rom bits of mucus in the stool. Ordinarily, however, special methods re required for isolation.

Bact. dysenteriæ (*Shiga*) is rather short with rounded ends and closely resembles he typhoid bacillus in gross morphological features. It does not possess flagella. t stains readily with the aniline dyes and is Gram-negative. It grows best at body emperature, is aerobic and facultatively anaerobic. It prefers a slightly alkaline ledium. On agar, broth, and gelatin growth resembles that of the typhoid bacillus. n litmus milk an alkaline reaction usually follows a slight primary acidity without ny further apparent change. On potato growth it is at first invisible but may appear ter of a brownish color. Acid is formed from dextrose, levulose, and galactose. Other types described differ from this in the fermentation of mannit and sometimes maltose.) Gas is never formed. Indol is not formed. (Other types usually form dol.) The toxins are probably chiefly endotoxins, though soluble poisons have also een demonstrated by some workers. The bacterium remains alive for months when reserved under the proper conditions. The thermal death point is 60° and re- stance to low temperature is considerable. It is sensitive to the usual strength of dinary disinfectants.

Dysentery does not occur in animals under natural conditions. By rtificial methods, however, it is claimed the disease has been repro- uced in dogs. Cultures, living or dead, are often extremely toxic to nall animals, especially the rabbit, and produce, after intravenous jection, violent intestinal symptoms, due evidently to the excretion an irritating poison. Nervous symptoms are also more or less arked and paralysis sometimes occurs before death. Immunity pro- uced artificially in animals is accompanied by the production of lysins nd agglutinins and lately antitoxins have been described in accord ith the demonstration of diffusible toxins. The agglutination in man of diagnostic value.

The epidemiology of dysentery is the same as for typhoid fever. In few instances the bacilli have been demonstrated in the fæces of ealthy persons, and convalescents may remain carriers for several onths.

Some success has been recorded from the administration of animal mmune serums and has been attributed to both lytic and antitoxic tion. Active immunization as a means of prophylaxis does not seem be of much value.

Fowl Diphtheria*

This disease, popularly known as Roup, and in its later stage canker, is characterized by a grayish-yellow fibrinous exudate, called false membrane. which forms upon the mucous membrane of the eye nasal passage, mouth, pharynx and larynx.

Roup, or fowl diphtheria, may be caused by a number of differen organisms, among them the well-known *Ps. pyocyanea* (green or blu pus organism), *B. cacosmus* and other species which give rise to a com plex suppurative process. The pus formed is semi-solid, cheese-lik and yellowish-white in color without any tendency to become soft an liquid or to perforate the surrounding skin. The organisms have tendency to penetrate the deeper layers of the mucous membrane c sub-mucous tissues, and hence swabs or cultures taken from the fals membranes may not contain the causal microörganisms which ar retained in the depths of the tissues. Sections of membranes fro affected fowls show large numbers of pus cells, some regular masse débris of epithelial cells and bacteria, and thus they differ from th diphtheritic membranes of man.

The organisms mentioned above have been isolated from affecte fowls, not only in America but also by Hauser in Europe. Sever investigators have described other bacteria producing false membrane and there are a few who think that coccidia are associated with th disease.

Both *Ps. pyocyanea* and *B. cacosmus* are able to produce false mem branes when inoculated into healthy birds, typical croupous and diph theritic membranes in the mouth and eyes; tumors in the subcutaneou tissues, the contents of which are firm, cheesy and yellowish-whit purulent conjunctivitis, blindness, purulent ophthalmia, and cheese-lik exudations in the bronchial tubes. These indications are identical wit the symptoms of "roup."

The disease is of variable virulence, and is apt to become chroni especially in unhygienic surroundings, and in draughty, badly ventilate damp houses. A common cold is a predisposing factor, and favo the invasion of the organisms mentioned.

Treatment of severe cases is useless, and demands too much tim Diseased birds should be isolated and the buildings thoroughly disi

* Prepared by F. C. Harrison.

cted. Slight cases may be cured by a 2 per cent solution of potassium ermanganate, in which the bird's head is plunged for a few seconds. his treatment should be given twice a day and continued until all mptoms have disappeared. The most effective preventive is to keep wls in good sanitary conditions—in dry, clean and well-ventilated uses, free from draughts.

Besides the organisms mentioned, Loeffler has described the *B. phtheriæ columbarium*, and Loir and Duclaux the *B. diphtheriæ gallinum* as causing fowl diphtheria, but the diseases produced by these ganisms are very dissimilar from the well-known "Roup" of North merica. The Klebs-Loeffler bacterium of human diphtheria has no thogenic effect on fowls.

Glanders*

Bacterium mallei

Glanders is a very common and serious disease, most common nong equines. It is communicable to the human being by inoculation d by the same process may affect sheep, goats, and laboratory imals. Cattle are not susceptible.

Bact. mallei and the disease it produces are widely scattered over e civilized world wherever horses are numerous.

This infection produces a disease which may be acute or chronic cording to the virulence of the microörganisms and resistance of the imal. Mules and donkeys are less resistant than horses and usually ve the disease in more acute form.

The characteristic features of the disease produced are inflammatory anges of the lymph glands and lymph vessels, ulceration of mucous embranes, the tubercle, the farcy bud, the lymph cord, and the culiar, clear, viscid discharge. There is considerable fever in acute ses, much less marked or absent in chronic cases. In a very common pe of the disease there frequently occurs a destructive inflammation the nasal mucous membrane which results in ulcers and consequent sal discharge.

Glanders in man is rare considering the frequent opportunity for fection. There are usually inflammatory swellings with involvement

* Prepared by M. H. Reynolds.

of local lymph glands, and constitutional disturbances soon follow t local symptoms. Human glanders is to be always regarded as ve serious with a probability of fatal termination. Ulcers may devel in the nose or mouth with more or less discharge. Pustules appe involving the skin, and abscesses involve deeper structures in varic portions of the body.

The distribution of *Bact. mallei* in the animal body is shown by t most common appearance of its disease in the skin, subcutaneo tissue, mucous membranes, lymphatic system, lungs, liver, spleen a kidneys.

The etiological factor is a small bacillus with rounded ends kno as *Bact. mallei,* discovered by Loeffler and Schütz and several oth in 1882, and well demonstrated to be the specific cause of glanders.

Entrance is usually effected by way of a mucous membrane, f quently the intestinal; sometimes by inoculation. The period incubation seems variable and uncertain under natural infection, b in artificial inoculation with virulent cultures, is very brief.

Bact. mallei probably produces toxins in artificial media and al in body tissues; the well-known preparation called *mallein* may considered in this class awaiting more definite knowledge. Th toxin (mallein) produces a distinct reaction by inoculation into glander animals, but is practically non-toxic for healthy equines. So f as known *Bact. mallei* attacks the animal tissues as do many oth microörganisms, the harm resulting chiefly from bacterial toxins whi give the local tissue reactions leading to the lesions characteris of glanders.

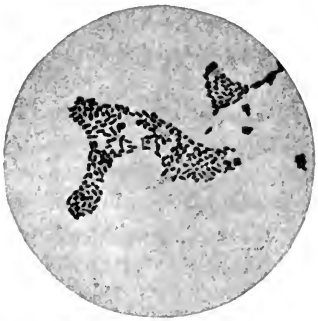
In its action on tissues *Bact. mallei* resembles *Bact. tuberculos* but shows a more rapid development of lesions and more active i flammation.

Lesions are of two types—a well-defined nodule followed by ulcer tion and areas of diffuse infiltration.

The nodule as it appears in glanders consists largely of lympho cells. Nodules die at the center, suppurate, and discharge. Th occurs especially in the external form of glanders, which affects mo commonly the legs and head. Occasionally defined enlargemen appear in the involved lung areas. Pulmonary lymph glands a frequently enlarged, and hardened. The superficial skin lesions a in the form of nodules previously mentioned, which usually suppura

nd ultimately heal. In the deeper subcutaneous tissues there is a endency to abscess formation. Small nodules or tubercles commonly ppear in the lungs of affected horses. These vary in size from millet eed to as large as garden peas. Various degrees of broncho-pnuemonia ppear and more or less pleurisy.

Bact. mallei shows no flagella and is non-motile. It is a small bacterium 0.25μ to .4μ thick by 1.5μ to 3μ long with rounded ends (Fig. 161). Spores have not been emonstrated. It is generally single. Coccus forms sometimes appear and even nort threads when grown on certain media; *e.g.*, potato. It decolorizes by Gram's ethod and is not easily stained by the aniline dyes. This bacterium grows fairly ell between 25° and 42° on potato, glycerin agar, or blood serum. The guinea ig gives a reliable diagnosis by inoculation, showing a diagnostic reaction within our or five days. Diagnosis may also be confirmed by the agglutination test in ilution of about 1:1000 or more and by the complement fixation test. Satisctorily stained in tissue section by Kuehn's carbol-methylene blue. Its growth is mited at an upper range of about 42° and the culture is killed at 55° in about five inutes. *Bact. mallei* is difficult to isolate by culture methods being a slow grower nd easily lost beside faster growing organisms. It can be better isolated by guineaig inoculation. In growth it is both aerobic and anaerobic.



IG. 161.—*Bacterium mallei.* From pure culture on glycerin agar. × 1000. (*From Migula.*)

The virus escapes from the body in various ways. Elimination s most common in morbid discharges from the nose, pharynx, trachea, nd in pus from farcy buds and abscesses.

Bact. mallei may be spread directly from the diseased animal to the usceptible animal, or the dissemination may be by way of intermediate objects; *e.g.*, troughs, feed boxes, water pails, etc., and perhaps lso by inhalation.

In man, infection occurs usually by inoculation. Cases produced i this way, occasionally appear among laboratory workers.

Bact. mallei is to be regarded as purely parasitic and limited in it natural activities to the animal body.

Bact. mallei is easily destroyed by a variety of unfavorable condi tions. It is destroyed by drying in fifteen to twenty days and is easil killed by heat or antiseptics.

All plain cases of glanders in domestic animals should be promptl destroyed. Exposed horses should be tested with mallein. Those tha react should be destroyed or quarantined, and contaminated premise properly disinfected.

Influenza*

Bacterium influenzæ

The natural disease appears to be limited to man. The incubatio period is very uncertain—probably very variable.

While the clinical manifestations of infection with *Bact. influenz* are variable the ordinary form begins with sudden severe headache accom panied by great prostration, pains and aches in the back and bones and a rapid rise in temperature. The fever lasts from three to fiv days and leaves the patient extremely weak, and depressed in bot mind and body.

Pfeiffer in 1892 described a bacterium which occurred in large num bers in the purulent bronchial secretion expectorated by influenz patients. The causal relationship has been quite definitely established

An examination of the sputum furnishes good presumptive evidence but cultivation is necessary for positive diagnosis.

The purulent material is streaked out in a drop of sterile bloo upon an agar plate.

Agglutination and complement fixation tests are valuable means fo identification.

In pure cultures the bacterium is 0.2μ to 0.3μ wide by 0.5μ long with occasiona threads up to 2μ in length. The ends of the rod are rounded. The arrangement i usually single, occasionally in pairs end to end and rarely in chains. The bacterium is non-motile and does not show capsules or spores. It does not stain readily wit ordinary aniline dyes. It is Gram-negative. Polar staining is shown sometimes

* Prepared by Edward Fidlar.

The temperature range of the influenza bacterium is about 26° to 41°. It is aerobic. It grows on artificial media only in the presence of blood pigment. On plain agar, glycerin agar, or blood serum with a thin layer of rabbit or human blood the colonies in twenty-four hours are very small, round and transparent, and remain discrete unless very thickly sown. The center of older colonies may acquire a yellow-brown color. In blood broth growth occurs quite readily if the medium is used in thin layers. It shows very much less resistance than the majority of non-spore-bearing bacteria. It is destroyed at 60° within about one minute. It is especially sensitive to drying.

The bacterium gains entrance through the mouth and nose and finds suitable soil in the mucous membrane of the respiratory passage. The toxæmia is due to absorption from this site rather than to the presence of the bacteria in the blood.

The bodies of the bacteria are distinctly pyogenic.

Secondary infections are prone to follow upon influenza so that abscess, gangrene of the lung, and empyema are not infrequent.

Influenza bacteria have been found in pneumonia, otitis media, and meningitis.

Immunity after natural infection is transitory.

The organisms are eliminated through the gates of entrance. Infection is for the most part direct, and follows close contact with a patient or carrier.

The bacteria are said to persist for many years in the bronchial secretions of convalescents and healthy individuals.

Therapeutically there is no specific measure for the control of this disease. Remembering that the infective agent is ejected during the coughing and speaking of the patient and is present in great numbers in the sputum, personal hygienic measures in both patient and contact should prove very effective.

Whooping Cough*

Bacterium pertusis

Whooping cough accounted for the death of 4,856 children in the United States in 1907. The causative agent, according to Bordet and Gengou, is an influenza-like bacillus.

It is a non-motile coccoid bacillus, stained faintly by aniline dyes and Gram-negative. It is distinguished from the influenza bacillus by

* Prepared by Edward Fidlar.

agglutination and complement deviation tests and by the fact that it can be gradually adapted to ordinary media.

The production of *pertussis* in young animals has been claimed. The organism has an endotoxin which produces local necrosis after subcutaneous injection.

Further evidence on the etiology of whooping cough is afforded by the observations of Mallory and others who have found large numbers of small microörganisms corresponding morphologically with *Bact. pertussis* occurring between the cilia on the epithelial cells lining the respiratory tract in fatal cases of the disease.

Hæmorrhagic Septicæmia‡

Bacterium bovisepticum

Hæmorrhagic septicæmia belongs to a class of similar diseases grouped under the general head of *Pasteurelloses*.

This disease has been reported from many portions of North America, from some sections of South America and many European countries. It is known under a variety of names, as cornstalk disease, buffalo disease, pneumo-enteritis, etc.

Bact. bovisepticum produces a serious disease and affects a wide variety of domestic and wild animals. The domestic animals most commonly affected are cattle, sheep, and goats; the disease being much more common among cattle than among other classes of stock.

The period of incubation in the artificial disease appears to be short, six to forty-eight hours. The onset of disease is usually sudden, and the case acute. Hæmorrhagic septicæmia does not spread from herd to herd but appears in isolated outbreaks usually at wide distances apart. It is a common experience to find a serious outbreak in one herd without any appearance of the disease in another herd in an adjoining pasture, with only a barbed wire fence between. Apparently the virus exists locally and, under as yet unknown conditions of increased virulence or lowered resistance, is able to start a local outbreak.

Hæmorrhages found at autopsy constitute the most specific and characteristic clinical evidence of this disease. Its mortality is very high, running from 50 to 90 per cent.

‡ Prepared by M. H. Reynolds.

Hæmorrhagic septicæmia of cattle, chicken cholera, and a number of other diseases belonging to this group are very similar in clinical features and the bacteria which cause these diseases are very similar in cultural and microscopic features. Yet all evidence points to the fact that *Bact. bovisepticum* acts as a specific causal agent for hæmorrhagic septicæmia of cattle.

Body infection probably occurs by both inoculation and ingestion. This disease does not appear to spread by simple association or ordinary contact and there is no general atmospheric distribution of *Bact. bovisepticum.*

Acute and rapidly fatal cases where the autopsy shows only trifling lesions would indicate the formation of active toxins. The characteristic hæmorrhages indicate the production of substances actively toxic for the endothelial cells of capillaries. The fact that these hæmorrhages vary in different cases from extensive subcutaneous areas to those that are scarcely visible would seem to indicate that this toxin is produced in greatly varying quantities or of greatly varying toxicity.

The lesions produced by this bacterium indicate a general distribution through the body.

The characteristic features as previously mentioned are the hæmorrhages which are either subcutaneous, submucous or subserous. Lymph glands are frequently infiltrated with extensive hæmorrhages.

Cases have been reported as showing high fever. Those studied by the writer have, as a rule, showed slight disturbance of temperature until near death. When voluntary muscles are involved the hæmorrhages invade connective tissues rather than muscle tissue proper.

Bact. bovisepticum resembles so closely the bacterium of chicken cholera, the bacterium of rabbit septicæmia, *Bact. suisepticus* and other members of this group (*Pasteurelloses*) that laboratory differentiation from other members of the group is exceedingly difficult. It is a very small bacterium with rounded ends, closely resembling a diplococcus. It is from 1μ to 1.5μ long and from 0.3μ to 0.6μ thick. Involution forms sometimes appear. It shows bipolar stain, decolorizes by Gram's method, produces no spores, has no flagella, and is non-motile.

The disease resembles anthrax in some general characteristics but is easily distinguished by microscopic examination of the blood and failure to find the large anthrax bacterium and by the fact that the blood from the general circulation is apparently normal in hæmorrhagic septicæmia. This disease also resembles symptomatic anthrax (black-

leg) but is easily distinguished in that external swellings are slight if present at all and do not show gas, both of these features being characteristic of blackleg. The bacillus of symptomatic anthrax may be recognized by microscopic examination as so different from *Bacterium bovi septicum* that there could be no mistaking one for the other.

Little is known concerning elimination of this bacterium from the diseased body and concerning methods of dissemination. Hence we are very much in the dark when attempting to deal with the disease produced by it.

Isolation and disinfection are to be recommended on general principles.

Leprosy*

Bacterium lepræ

Leprosy is a disease almost as old as history itself but modern leprosy cannot be definitely identified with the leprosy of the Old Testament, and to day is found chiefly in oriental countries and in Norway, Iceland and Russia. The disease is present in some of the provinces of Canada and in the States of Louisiana, California and Minnesota, and practically limited to Scandinavians in the latter states. The natural incubation period is difficult to ascertain but is probably a matter of months or years.

Clinically there are two main types of the disease, the tubercular or nodular and the anæsthetic types. In the first form, nodules develop in the face or other parts of the body usually preceded by an erythematous patch. The mucous membranes become affected more or less extensively and the hair and eyebrows fall out. In the anæsthetic type after various disturbances of sensation which may sometimes be followed by maculæ there develop areas of anæsthesia. Bullæ, ulcers and necrosis may occur with resulting deformities or again this type may exist for years without leading to such results.

The bacteria of leprosy were first described by Hansen in 1879 and almost at the same time Neisser published similar descriptions. Cultivation of *Bact. lepræ* has been successful in the hands of Clegg, Duval and others.

The microörganisms can be shown in tissue by the use of the Ziehl-Neelsen or Gabbet methods.

* Prepared by Edward Fidlar.

In tissue the bacterium closely resembles the bacterium of tuberculosis, but usually appears somewhat longer (5μ to 7μ) and thicker (about 0.5μ) straighter and less beaded. Flagella have not been demonstrated. The bacterium can be stained with the ordinary aniline dyes. It is Gram-positive. The staining reactions on the whole are like those of *Bact. tuberculosis* but *Bact. lepræ* stains more readily and also decolorizes more readily; 30 per cent nitric acid followed by 95 per cent alcohol will totally decolorize them while *Bact. tuberculosis* resists. The optimum temperature for growth ranges from 32° to 35° when grown in symbiosis with amœbæ. The reaction of the media upon which successful isolation takes place is 1 to 1.5 per cent alkaline to phenolphthalein. In recently isolated cultures growth is extremely slow and appears on the surface of the special media in four to six weeks as moist grayish-white colonies elevated centrally, with an irregular wavy margin and attaining a diameter of 2 mm. Older cultures on glycerin agar are moist and abundant, and develop an orange-yellow pigment. In glycerin broth a thin membrane is formed at the surface after several weeks, while a small amount of sediment collects at the bottom of the tube leaving the medium clear. The resistance to heat is much greater than that of ordinary vegetative bacteria, so that cultures may be freed from contamination by the latter by simply heating to 60° for one hour. The resistance to drying is probably considerable.

Human leprosy appears to be confined naturally to man and only lately has the disease been transmitted artificially to animals. In the Japanese dancing mouse, and less frequently in the white mouse and the monkey small nodules may be found on the peritoneum about four to eight weeks after intraperitoneal inoculation. The animals do not show any symptoms of illness and must be killed in order to find the lesion. More recently Duval has produced an apparently typical leprosy in monkeys by repeated injections of cultures from artificial media.

It is generally considered that the usual path of entrance of the bacterium is the naso-pharyngeal mucous membrane. The organisms seem to be distributed slowly over the body and according to their location produce the different types of the disease. They are found in the nodules of the nodular type and in the nerve trunks of the anæsthetic type.

Agglutinins have been demonstrated in the blood of lepers. Complement deviation with various antigens has been investigated and indicates an antilipoid immune body which not infrequently gives a positive Wassermann reaction. Lepers react frequently to tuberculin inoculations and this is not considered to be always due to associated tuberculosis.

The chief source of elimination of leprosy bacteria is the nasal

mucosa. The bacteria have been demonstrated in this region in abou 40 per cent of the macular types, 80 per cent of the nodular and mixe types.

While a great deal of popular fear exists against this disease it i decidedly less infectious than pulmonary tuberculosis. Lepers hav unquestionably been subjected to a great deal of wholly unnecessar persecution.

Prophylactically, isolation has certainly demonstrated its valu and the reported increase of leprosy in certain parts of Europe has bee attributed to a decrease of this custom of segregation.

Plague*

Bacterium pestis

Epidemics have been recognized since the second century. Abou half the population of the Roman Empire died in the sixth century An epidemic of the fourteenth century destroyed half the inhabitant of Europe. In India during 1901 to 1904 about 2,000,000 died of the disease. In China, in Egypt, South Africa, and in sea ports of the Western hemisphere, plague has been found.

Among animals the disease has been found chiefly in rats and squirrels. Dogs may occasionally become infected.

Four types are described, the ambulant, bubonic, septicæmic, and pneumonic. The bubonic type forms three-quarters of the cases. Physical and mental depression accompanied by a high fever, often with a remission about the third day, occurs. Collapse may then follow with death. Glandular swellings (buboes) appear in the groin and axilla and these may suppurate. Hæmorrhages beneath skin and mucous membranes are common. The third type is a very rapid form, causing death before the development of buboes. The fourth type is also a short and extremely fatal form, marked by the occurrence of broncho-pneumonia due to the plague bacteria.

The bacterium of bubonic plague was described by Yersin and Kitasato independently in 1893. They found it in glands and throughout the body in fatal cases.

The organism is readily grown from the buboes, the blood, and the sputum in the pneumonic type, by simple inoculation of ordinary media of a slightly alkaline reac-

* Prepared by Edward Fidlar.

ion. The bacteria are 1.5μ to 1.7μ long by 0.55μ to 0.7μ wide with rounded ends ccurring singly or in pairs and short chains in exudates and sometimes in long hains in broth. Involution forms, large swollen spheres, clubs, etc., are characteristic in artificial media. There are no spores. It is non-motile. Some bservers have demonstrated a gelatinous capsule. Occasionally very distinct ranching occurs (Hill). It stains easily with aniline dyes, particularly at the oles which may show round or oval granules. It is Gram-negative. Its minimum emperature for growth is about 12°, the optimum 30°, the maximum 40°. It is

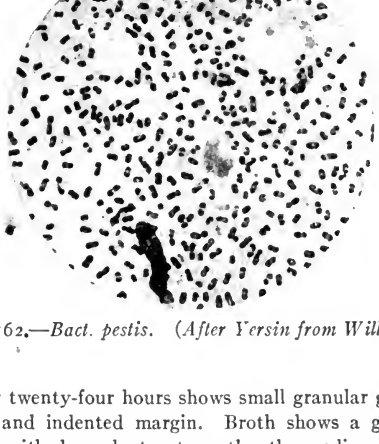


FIG. 162.—*Bact. pestis.* (*After Yersin from Williams.*)

robic. Agar after twenty-four hours shows small granular grayish colonies with thickened center and indented margin. Broth shows a granular deposit and metimes a pellicle with dependent outgrowths, the medium remaining otherwise ar. Gelatin growths are as on agar, and the medium is not liquefied. Litmus lk may show slight acid formation and no coagulation. Potato shows nothing aracteristic. The toxins appear to be largely endotoxins, though soluble poi- ns have been found in old cultures. No indol is formed. Resistance toward at is not great, boiling kills in a few minutes. Light kills in a few hours. They not resist drying well, but in a moist condition remain viable for over a year. e usual strengths of ordinary disinfectants kill in about ten minutes.

Rats, mice, guinea-pigs, rabbits, and monkeys are particularly sceptible to inoculation and even insects die from infection.

The bacterium enters the body through the (usually abraded) skin respiratory tract. After involvement of the nearest lymphatic nds the bacteria are distributed through the blood.

Single attacks immunize. The antibodies developed are agglutinins,

probably bacteriolysins, and possibly antitoxins. The agglutinatio reaction is of value in diagnosis.

The organisms are eliminated in the exudates from suppuratin buboes, in the sputum in the pneumonic type, and are present through out the body after death. The dead bodies of human beings and o rats are sources of infection for other rats. There seems good evidenc of these animals becoming chronic carriers though showing no symp toms of disease, and may thus be important factors in maintaining an spreading plague.

The disease is largely communicated by means of fleas which hav become infected by living on other human beings or even upon rat

Prophylaxis consists of isolation of pneumonic cases, thoroug disinfection involving the killing of fleas, and chiefly the destruction c rats, squirrels, and other animals which may serve as carriers. Haf kine's vaccination method has also been shown to be a valuab prophylactic measure.

The serum of immunized animals has been tried as a therapeut agent and gives encouraging results when administered in the earl stages.

Swine Erysipelas*

Bacterium rhusiopathiæ suis

Swine erysipelas is an infectious disease of hogs characterized b red or violet discoloration of the skin and mucous membranes. Swi erysipelas does not exist in the United States but is very prevalent continental Europe. It is caused by a very small, slender, non-motil non-spore-bearing bacterium (*Bact. rhusiopathiæ suis*) which stains b Gram's method, and grows feebly on the ordinary culture medi Development is best under anaerobic conditions. In gelatin st cultures, after three or four days, a white growth can be seen alo the needle puncture. Radiating from this are a number of delica tufts which give the growth the appearance of a fine test-tube brus White and gray mice, white rats, and pigeons succumb to the inocul tion of minute amounts of the culture. The bacteria tend to colle within the bodies of the leucocytes. This microörganism is close related to and possibly identical with the bacterium of mouse septicæm

* Prepared by M. Dorset.

act. murisepticum). Preventive inoculation with attenuated cul-res has long been practised successfully in Europe.

TUBERCULOSIS*

Bacterium tuberculosis

Consumption, phthisis, scrofula, pearl disease, etc., are synonyms the term tuberculosis.

This bacterium in its several varieties produces a very universal ease; practically all common animals and man are subject to it. ttle and swine among the domestic animals are especially susceptible this infection and wild animals in captivity easily become affected.

The normal progress of tuberculosis is slow. Its characteristic ture is the tubercle or nodule of various sizes.

Tuberculosis is probably the most common and serious of all diseases either animal or man.

In 1906, 138,000 persons died from tuberculosis in the United States, at the rate of 164 per 100,000 population. Based upon these facts,† is estimated that about 5,000,000 of those now living in the United Stes may die of the disease. It is claimed that the disease alone costs t United States from $400,000,000 to $1,000,000,000 each year (sher).

If the loss from wage earnings, the cost of the patient in suffering, dical treatment, medicines, nursing, board, and care, also the suffer-i and sacrifice entailed by near relatives, friends, and communities a considered, the loss to the country mentioned above does not appear enormous.

It is estimated by the United States Bureau of Animal Industry that 2 er cent of hogs in the United States are tubercular, and that losses of stk in the United States, due to tuberculosis, amount to $23,000,000 a ually. Of 400,000 cattle tested in the United States prior to 1908 95 per cent were tubercular. The highest prevalence of tuberculosis i attle is among pure bred herds and in city dairy stables; *i.e.*, among th cattle kept most closely confined. It is most common in old cattle a rare in calves under six months old. Tuberculous infection is quite g erally scattered among cattle of civilized nations.

Prepared by M. H. Reynolds.
1911.

Tuberculosis appears in man usually in the form of lupus (tuberc losis of the skin), or scrofula (tuberculosis of the cervical glands), phthisis (tuberculosis of the lungs). It also frequently appears in t mesenteric glands and other glands of the body, and may appear in a of the tissues. It is quite possible, judging from autopsies, that ma persons have tuberculosis without realizing its existence in the bod and without its being detected in any way. It is questionable, ho ever, whether under such circumstances the disease is transmitted disseminated.

As a rule affected cattle show no definite outward signs of the d ease. Badly diseased animals frequently appear poor and thrif Many cases are mild and latent. A few tubercular animals cou some show harsh hair and skin and other expressions of ill heal While these symptoms do not necessarily indicate tuberculosis, th are very suggestive.

Bact. tuberculosis may invade almost any tissue or organ of man the animal body and produce a variety of lesions. Man usually gi some evidence of the disease either objectively or subjectively, and many instances the disease assumes a definite form which is easily r ognized by medical men, unlike its presence in animals. The sym toms are more evident in swine than in cattle. Affected hogs are of unthrifty and show glandular enlargemets and degenerations of t enlarged glands.

Avian tubercle bacteria are becoming disseminated among poult and to a serious extent in some sections of the country. Among more prominent symptoms of avian tuberculosis are emaciation w marked anæmia and weakness. Examination of the carcass shows d ease most frequently in the liver, but intestines, spleen, lungs, and e the skin may be invaded. The avian tubercle bacterium varies certain respects from the bovine variety; it usually is shorter, measur from 1μ to 4μ in length with a general average of 2.7μ and grows b at higher temperatures. Danish authorities report* serious outbre among swine due to avian tubercle bacteria.

It has long been firmly established that *Bact. tuberculosis* is the s cific cause of this disease. But while this bacterium is to be regar as the specific cause it must be understood that this organism is quently associated with pus-producing bacteria which are responsi

* Dunne, Trans. Jour. Bd Agr. (London), 22 (1915), No. 1.

r certain phases of the disease as commonly seen. It should be under-
ood also that persons and animals become more susceptible and have
eater opportunities for infection under close confinement and lack of
ercise. There has been great difference of opinion concerning the
ity of the tubercle bacterium, and the probability of inter-trans-
ssion between man and the lower animals. A large number of bac-
iologists now hold that the several types of tubercle bacteria are but
vironmental variations of the same species. In any case, man clearly
pears susceptible to both human and bovine types at least.

The entrance of the germ may occur in four ways, namely, by way
the digestive tract; it may occur by way of the respiratory organs;
may occur by inoculation; and infection may possibly occur before
th. Some authorities hold that the most common infection is by
y of the digestive tract and in early life. Others hold that inhalation
berculosis is most common.

This bacterium produces a slow toxæmia, and it is this toxæmia to-
her with physical embarrassment of the vital organs by extensive
ions which together harm the affected body. Various toxins are
oduced, as indicated by the fact that killed cultures by subcutaneous
ection may destroy local tissues and produce abscess, debility, and
aciation. Production of toxins is indicated by the further fact that
tain antitoxic immunity may be produced by minute doses of killed
ture gradually increased.

Tuberculin is a common and well known product or mixture of pro-
cts produced by this bacterium. One of its constituent products is a
er producer. Another product has been reported which reduces tem-
ature, and still another which produces convulsions, in sufficient dose.

Tuberculosis may be very general. Almost any tissue or organ in
body may be invaded; but as a rule, not many organs are badly
ected in the same case. Distribution occurs by way of both the blood
lymph streams, especially the latter. It seems probable that tuber-
bacteria may be distributed in the body by wandering phagocytes.

The *Bact. tuberculosis* has a characteristic tendency to produce tuber-
or nodules which may be large or small and which have a tendency
central necrosis and degeneration. Mucous membranes, under this
ection, tend to develop superficial ulcers.

The lesions produced by this microörganism may vary from the
iest tubercles to extensive areas of large organs. Lymph glands

frequently enlarge and undergo cheesy or calcareous degeneratio Tubercular masses of various sizes may appear upon the lining mer branes of the chest and abdominal cavities and upon various intern organs. Cheesy abscesses may appear in the depths of soft orgar In cows the udder is occasionally enlarged and shows hard mass with little or none of the heat usually occurring in connection with i flammatory changes. Bones and joints are often involved especial in the human; these structures increase in size, produce pain, a suppurate.

Bacterium tuberculosis is a slender rod-shaped organism with rounded ends a under certain conditions shows granular forms. It varies between 2μ and 5μ length, and 0.3μ to 0.5μ in width. This bacterium is usually straight, but may bent; it appears either singly or in groups or branched, non-motile, and is probal not a spore producer (Fig. 163). Glycerin agar, blood serum, egg slant, a bouillon may all serve as satisfactory nutrient media. It is aerobic and its te perature limits for growth appear to be 29°–42°C. With the exception of you and rapidly growing forms it is strongly acid-fast. Tubercle bacteria may

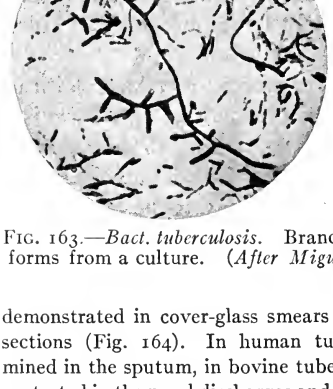


FIG. 163.—*Bact. tuberculosis.* Branching forms from a culture. (*After Migula.*)

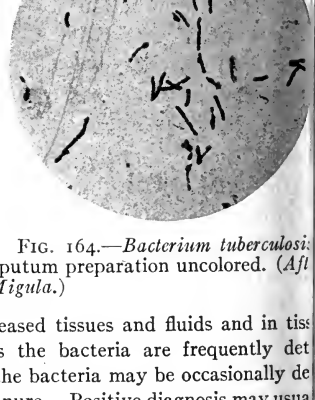


FIG. 164.—*Bacterium tuberculosis* Sputum preparation uncolored. (*Af Migula.*)

demonstrated in cover-glass smears from diseased tissues and fluids and in tis sections (Fig. 164). In human tuberculosis the bacteria are frequently det mined in the sputum, in bovine tuberculosis the bacteria may be occasionally de onstrated in the nasal discharges and in the manure. Positive diagnosis may usua be made by guinea-pig inoculation. For microscopic examination a cover-gl smear is fixed in the usual way, then stained with hot carbol-fuchsin three to f minutes or in cold stain fifteen to twenty minutes. It is then decolorized, e in 10 per cent nitric acid, and counterstained with methylene blue for about c minute, after which it is rinsed and ready for examination.

It is conceded that tubercle bacteria do not multiply in nature outside the animal body and, therefore, dissemination must depend wholly upon the dissemination of infected people or animals and materials infected by diseased men and animals. Tubercle bacteria escape from open ulcers or from tubercular lesions which connect with digestive or respiratory organs. They may reach the surface in other ways; *e.g.*, by the discharge of abscesses.

In controlling tuberculosis among humans at the present time, several methods are in vogue. In some localities, an effort is made to segregate tuberculous patients during the day for the purpose of treating them as well as teaching them how to care for themselves. This method aims to instruct how to prevent dissemination and transmission of the disease, to prepare suitable nourishment, and to secure the advantages of open-air influences. This instruction not only extends to the patients but others with whom the patients may mingle. Sanitaria are also constructed to receive patients suffering from the disease, and care for them under suitable medical supervision by proper treatment, nourishment, and open-air life. Again, the policy is being inaugurated to instruct tuberculous patients, where it is impossible to reach them by other means, to care for themselves in their own homes.

By these general hygienic measures, much good has been accomplished, not only for the patients but, also, in a diminution of the number of new cases developing.

The animal disease is carried to distant points, most commonly by breeding stock. Locally the disease spreads either by the movement of affected cattle, or frequently

FIG. 165.—*Bact. tuberculosis.* Glycerin agar culture. (*After Curtis from Stitt.*)

by infected milk. Hogs receive their infection from the milk of tubercular cattle or from the manure or carcasses of such cattle in feeding yards. Unventilated stables are favorable for the spread of this disease because with insufficient ventilation the bacteria are not carried out, but become constantly more numerous. The tubercle bacterium is quite resistant to drying, but is rather sensitive to sunlight. It is usually destroyed by moist heat in six hours at 55°; in twenty minutes at 60°; and generally in five to twenty minutes at 95°, depending upon the protection it may have.

Conditions of sensible sanitation are of the utmost importance. These include exercise, sunlight, and ventilation, particularly sunlight. In order that effective control work may be done among animals tuberculin must be used freely and conscientiously.

The method of dealing with diseased herds depends upon breeding and value. Common cattle are usually dealt with most economically and efficiently by slaughter with a view to using such carcasses as may pass inspection. Valuable cattle, especially pure bred animals, may be used for breeding purposes, gradually building up a sound herd and gradually displacing the diseased animals. This latter plan is usually unprofitable and unwise except for valuable cattle.

Foot Rot of Sheep*

Bacillus necrophorus

This is an infectious disease of sheep characterized by an ulcerative inflammation of the tissues just above the horny part of the cleft of the hoof. It is seen in Europe, England, Australia, and the United States. Sheep are made lame and if the disease is not checked by appropriate treatment, the hoof becomes greatly distorted, the sheep being finally unable to walk. Mohler and Washburn† state that foot rot is caused by *B. necrophorus*, this organism being associated with pus-producing micrococci. *B. necrophorus*, which is a strict anaerobe, tends to grow out into long filaments; it is stained by the ordinary aniline dyes, but not by Gram's method. Rabbits and white mice are susceptible to inoculations of this bacillus, but guinea-pigs appear to be immune. The disease is treated by causing infected sheep to walk through a disinfecting solution, such as a 3 per cent solution of carbolic acid.

* Prepared by M. Dorset.

† Bull. 63. Bur. An. Industry, U. S. Dept. Agr., 1904.

Malignant Œdema*

Bacillus œdematis maligni

The disease occurs as the result of infection of wounds with dust or oil. The wounds must involve the tissues deeply as in compound ractures and deep cuts.

Any animal may be infected, although the dog and cat are said to be ather more resistant than others.

The incubation period is short, from one to two days as a rule.

The usual case begins with sudden spreading hæmorrhagic sub-tances, œdema and high fever. Practically no gas is formed. The uid shows bacilli both with and without spores. Where soil contami-ation exists, mixed infections with gangrene are common.

Pasteur in 1877 and Koch and Gaffky in 1881 found and studied ie organism and by passing from animal to animal established the ausal relationship.

Glucose agar or glucose gelatin is inoculated with the suspected fluid, plates oured and placed under anaerobic conditions. The organism is 0.8μ to 1μ wide. ilaments may occur. The rods without spores are uniform in width with slightly uared ends. They are usually single, though pairs end to end are frequent and ains are also found. Oval spores are formed somewhat variable in their position, ith a diameter usually larger than that of the vegetative rod, bringing about a indle shape. Peritrichic flagella have been demonstrated, about twenty in mber. It stains readily with aniline dyes, usually Gram-negative though some-hat variable and indefinite in this regard. Growth takes place at both 20° and °. It is a strict anaerobe. Like anaerobes in general it prefers the presence of a rmentable carbohydrate such as glucose. On agar the colonies are small, whitish, d irregular in outline. Gelatin and blood serum are digested, caseinogen is anged to casein which is then digested. In both protein and carbohydrate media gas is produced which has a very disagreeable odor. The spores are very resistant.

This resistance accounts for its continuous presence in earth and ust and as a constant inhabitant of the intestine of animals, especially herbivora.

Milksickness†

Bacillus lacti morbi

Milksickness, a disease so called because of it being most commonly ansmitted to man through milk of infected cows, is caused by the

* Prepared by Edward Fidlar.

† Prepared by Norman Mac L. Harris.

growth of and consequent absorption of toxic principles from the infective agent lodged primarily in the small intestine, and secondarily in the liver and other organs of the body.

The disease appears to be peculiarly an American one, and has been reported at intervals for many years from several of the Middle Western States and Kentucky, Tennessee, Virginia and North Carolina, and more recently from New Mexico. The bacterium, *Bacillus lactimorbi*, regarded by Jordan and Harris as the causative agent, appears to flourish chiefly in the moist, shaded pasture lands of creek bottoms or, as in New Mexico, on pasture lands that are subject to irrigation or flooding.

Bacillus lactimorbi is an aerobic, spore-bearing, non-liquefying, non-gas-forming microörganism, which, when stained with Loeffler's methylene blue, shows one or more metachromatic granules within its body. In young cultures it is Gram positive, slowly alkalinizes milk, which at times becomes clarified through the great accumulation of hydroxyl ions. Agar plate colonies resemble those of streptococcus and there frequently is developed a delicate film growth over the plate. It, or members of its group, are seemingly widely distributed in nature, as Luckhardt has found bacteria practically identical with *Bacillus lactimorbi* on alfalfa obtained from Wisconsin, Illinois and Indiana, as well as on plants of several species from New Mexico. Certain of these strains possessed pathogenic properties, others showed none; therefore, it is believable that the bacterium may, under peculiar environmental conditions, develop varying degrees of virulence and pathogenicity. It may be properly regarded as a facultative pathogen.

Pathogenicity is demonstrable for young rabbits, guinea-pigs, dogs, cats, lambs, and calves, although it must be borne in mind that its virulence at any time is of low degree. Infection, either through natural or artificial channels, occurs only after considerable quantities of infective material are swallowed. In man, the disease is progressively marked by lassitude, loss of appetite, nausea, gastric pains, vomiting, obstinate constipation, a fall in body temperature, odor of acetone on the breath, the presence of acetone, diacetic and β-oxybutyric acids, and the *absence* of dextrose in the urine. Large doses of sodium bicarbonate are relied on to effect a cure. In the herbivora, muscular weakness, tremors, constipation, odor of acetone on the breath are prominent features; in horses, profuse sweating may be a prominent symptom. Degenerative and fatty changes of the parenchymatous organs is the especial pathological feature met with.

Eradication of the disease in localities where it occurs may be

effected by, either the cultivation of the infected pasturage, or by securely fencing it in.

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Symptomatic Anthrax or Blackleg*

Bacillus anthracis symptomatici

Blackleg, black quarter, symptomatic anthrax, quarter ill, are synonyms employed to designate this disease.

Symptomatic anthrax is a very old disease and until recent years has been confused with true anthrax. This disease is widely distributed, affecting practically all countries and climates.

It is enzootic, never spreading widely or rapidly, and is often found in certain infected valleys and in relatively small areas. Young cattle, generally under two years of age, are most commonly affected, but sheep and goats are susceptible to this infection.

This disease is infectious by inoculation, perhaps also by ingestion, and usually acute. Subcutaneous and muscular tissues are especially affected. Its most prominent and characteristic feature is swelling, affecting most frequently, the front or hind quarters, and never extending below the knee or hock. As a rule, the bacillus of symptomatic anthrax produces a very acute disease with severe constitutional disturbances.

The bacillus of symptomatic anthrax has been clearly demonstrated to be the specific cause of blackleg, infection occurring by inoculation. The period of incubation in the natural disease is uncertain. Under artificial inoculation this period varies from two to three days and is occasionally as short as one day.

This bacillus produces in culture a very active toxin. This toxin is quite resistant to heat. That the bacillus of symptomatic anthrax stimulates the production of antibodies and that the injury is done by

* Prepared by M. H. Reynolds.

toxins, is shown by the fact that immunity against virulent culture may be produced by treatment with presumably sterile filtrates of virulent cultures.

The bacillus of symptomatic anthrax is rarely found in the general blood before death; but is abundant in the affected muscle and overlying subcutaneous tissue. It also occurs in great numbers in the bile and intestinal contents.

Mucous membranes become congested and then very dark. There is a high fever. Local swellings occur which are at first sensitive and later insensitive and gaseous. There is usually developed a very marked swelling of a front or hind quarter or of the neck, with rapid formation of gas. The serous membranes, particularly the pleura and peritoneum, develop severe inflammation with hæmorrhages and infiltrations and corresponding exudation in the cavities. General decomposition is rapid and the swelling may show a slight acetone odor. The local subcutaneous tissues are infiltrated, hæmorrhagic or gaseous. The local lymph glands are swollen and hæmorrhagic or œdematous. Muscle fibers show various degenerative changes. The abundant gases are mostly hydrogen and carbon dioxide.

B. anthracis symptomatici is about 3μ to 6μ long by 0.5μ to 0.8μ thick. This is a spore-bearing bacillus of drum-stick shape or spindle shape and is anaerobic. It grows best at about 37°. It stains either by the simple aniline dyes or by Gram's method. In artificial cultures, it sometimes shows long forms. This organism is motile for a short time, but soon loses this power, probably on account of the oxygen to which it is exposed. It shows well-defined flagella and develops spores. The specific organism may be demonstrated by the microscope in the blood without staining if done soon after death.

The bacillus of symptomatic anthrax is easily demonstrated in cover-glass smears from the affected tissues, and is very different from the bacteria of anthrax and hæmorrhagic septicæmia, the only diseases liable to be mistaken for blackleg excepting malignant œdema Anthrax gives a surface growth and is aerobic. Symptomatic anthrax gives no surface growth and is anaerobic. This organism may also be demonstrated by animal inoculation. The guinea-pig serves well for this purpose; it is very susceptible to inoculation and gives a characteristic blackleg reaction in both symptoms and lesions. From the lesions thus produced the characteristic bacilli are easily demonstrated by the microscope.

Elimination of this virus from the body occurs chiefly in the various discharges, and especially in the manure and also in general decomposition of the carcass. Dissemination of this disease is chiefly if not exclusively by diseased carcasses and parts of carcasses and by the discharges. Contaminated soil plays a very important part in the prevalence of this disease. It appears probable that the specific bacillus may even multiply in the soil.

Carcasses should be burned if possible; otherwise very deeply buried and covered with lime. Contaminated grounds, or stable floors must be thoroughly disinfected, for the infection is very persistent and difficult to eradicate except by most vigorous effort since the spores are very resistant to heat and drying. Preventive inoculation after the method of Arloing and Kitt is very satisfactory. Their vaccine consists of specially treated muscular tissues from the diseased part.

Tetanus*

Bacillus tetani

This disease is found throughout the world but more frequently in warmer than in colder climates. Certain localities are particularly affected. Man and domestic animals are susceptible.

The incubation period varies from a few hours in experimental inoculation of small animals, to several days or weeks in cases of natural infection in man.

The disease follows a wound of a punctured type with contamination by earth, especially in injuries of hands and feet.

It is characterized by tonic spasms of the voluntary musculature usually beginning in some one group of muscles and finally becoming general. The parts first affected are, in cases artificially produced, those at the site of inoculation, but in natural infections in man it is more common for the disease to manifest itself by stiffening of the muscles of the neck and face, producing what is ordinarily termed "lockjaw." In less severe infections in man local pain and stiffness are the first indications. The spasms occur in paroxysms which are spontaneous or excited by effort. They are more or less prolonged and exhausting and are accompanied by greater or less pain. Death results

* Prepared by Edward Fidlar.

from general loss of strength or involvement of the respiratory muscles. The shorter the incubation period the higher the mortality. Few recover when the incubation period is less than ten days, about half the cases recover when the period is more than fifteen days.

The nerves may show injury as indicated by swelling and redness and microscopically nerve cells have been observed in a state of granular degeneration; there is a more or less distinct general congestion of the organs.

While lockjaw has been known clinically for centuries, it was not until 1884 that the infectious character was demonstrated when Carlo and Rattone and Nicolaier were successful in animal inoculations. Kitasato obtained pure cultures of the bacillus in 1889.

The organisms may be detected occasionally by examination of stained preparations of the pus from the wound. Pure cultures may be obtained by inoculating an alkaline dextrose broth with pus or tissue, incubating under anaerobic conditions for about forty-eight hours until sporulation, then exposing half an hour to a temperature of 80° to kill all vegetative forms and subsequently making subcultures. If other spore-bearing bacteria are present considerable difficulty may be encountered. Subcutaneous inoculations of mice or guinea-pigs is a good method for demonstrating the presence of the organism, but pure cultures should be combined with some aerobe (say *B. coli*) to secure results.

The *B. tetani* is about 2μ to 5μ in length by 0.3μ to 0.8μ in width with rounded ends. The vegetative rods are uniformly cylindrical but the terminal spores give a "drum stick" appearance (Fig. 166). The arrangement is usually single, but threads may occur especially in old cultures. The organism forms round terminal spores which have a diameter of 1μ to 1.5μ. The young bacilli are motile and possess 50 to 70 peritrichic flagella. Motility is lost with sporulation. The bacillus is stained by the aniline dyes and is Gram-positive. The spores are readily demonstrated by the special stains. The range of temperature for growth is from about 14° to 45° with an optimum about 37°. The organism is usually considered an obligate anaerobe though experimentally aerobic strains have been developed but with loss of pathogenic and toxogenic properties. Pure cultures do not develop in an atmosphere of carbon dioxide. Media for the cultivation of the bacillus should be slightly alkaline and should contain for best growth about 2 per cent of glucose or 1.5 per cent sodium formate. The addition of pieces of fresh raw sterile tissue is valuable. On agar at 37° colonies appear in forty-eight hours which show microscopically, a mass of tangled threads resembling colonies of *B. subtilis* or *Bact. anthracis*. In broth a cloudiness is produced in twenty-four to

thirty-six hours with the development of gas and a very disagreeable odor. In gelatin the colonies develop more slowly than on agar and show liquefaction. In old stab cultures a pine tree growth occurs. Gas is usually produced. In milk growth occurs without coagulation. Acid is produced in some carbohydrate media. Gas is produced during the action upon protein and consists chiefly of carbon dioxide but also of hydrogen sulphide and certain volatile organic compounds commonly found in putrefactions. The tetanus bacillus forms two soluble toxins, tetanolysin, and tetano-spasmin. The former is less stable and dissolves red blood-corpuscles. The latter produces the characteristic spasms of the muscles. This poison may be obtained after one to two weeks growth in slightly alkaline salt-peptone-bouillon under anaerobic conditions at 37.5° and separated by filtration

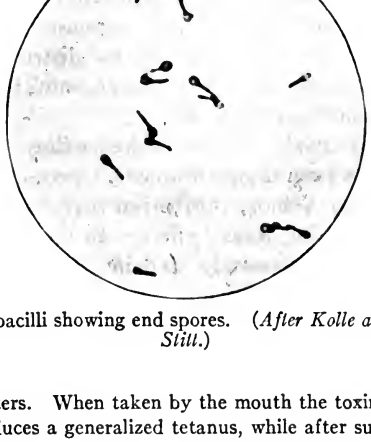


FIG. 166.—Tetanus bacilli showing end spores. (*After Kolle and Wassermann from Stitt.*)

through porcelain filters. When taken by the mouth the toxin is ineffective, given intravenously it produces a generalized tetanus, while after subcutaneous injection the disease begins with local spasms. The central nervous system is reached by ascent of the toxin along the motor nerves nearest the point of inoculation. A dose of toxin injected directly into the nerve trunk of an animal may produce a fatal result when it is innocuous intravenously. The spores often withstand 80° for one hour and live steam for about ten minutes. Direct sunlight destroys them in time. They survive drying for several years and resist the ordinary disinfectants for a considerable length of time, 1:1000 mercuric chloride for three hours, 5 per cent carbolic acid for about ten hours.

Practically all mammalia are susceptible to tetanus though rats are but slightly so. Very minute doses of toxin suffice to kill mice and guinea-pigs. Birds show but little susceptibility and the hen is said

to be three hundred thousand times more resistant to tetanus toxin than the horse. Reptiles and amphibians are practically immune to very large doses when kept at low temperature.

Natural infections probably do not occur without the presence of other microörganisms. The bacillus and its associated material gain entrance through some break in the tissues. The organism is practically confined to the site of inoculation, but it is sometimes found in the blood and internal organs after death.

Against toxin-freed cultures phagocytosis is probably the process which overcomes infection. The toxin is highly antigenic and animals can be immunized against it in a manner similar to that for diphtheria toxin.

While direct infection of one person from another has occurred, cases of human tetanus are very rarely responsible for others.

Horses are chiefly responsible for its distribution, the tetanus bacillus being common in manure, which accounts for the occurrence of tetanus in soil-contaminated injuries.

Cattle probably are also carriers of the bacillus. Tetanus antitoxin as a cure has been a keen disappointment, especially if symptoms have fully developed after a short incubation period. Very large dosages, however, may have the desired effect. In prophylaxis the antitoxic serum is widely and successfully used in all suspicious cases, and in Fourth of July injuries in particular.

Typhoid Fever*

Bacillus typhosus

Typhoid fever is one of the most widespread of bacterial diseases and is found endemic in practically all the countries of the world. Epidemics frequently occur because of the infection of some local public utility related to food or drink, particularly water or milk.

Typhoid fever occurs naturally only in man. Intraperitoneal inoculation of susceptible animals may result in death with acute peritonitis, but lesions are in no way specific and can be produced by the colon bacillus.

* Prepared by Edward Fidlar.

The period of incubation varies ordinarily from five to twenty-one days, with an average of fourteen days.

The first week of the disease in man begins with a train of rather indefinite symptoms such as headache, loss of appetite, digestive disturbances, lassitude, and sleeplessness. Nose bleed is a peculiar and rather constant feature. The temperature and pulse gradually rise until by the end of five to seven days the former has become high, 103°F. to 104°F. and constant. The temperature continues thus through the second week during which a gradual stupor and occasional delirium, diarrhoea, and enlargement of the spleen occur. The pulse is often dicrotic and there is a rash consisting of isolated flattened rose-colored macules or spots which may be few or numerous and occur in successive crops. During the third week in mild cases these symptoms gradually subside. In severer forms no abatement is shown and complications are liable to occur. The fourth week shows beginning convalescence in the typical case.

The characteristic pathological findings are swelling and ulceration of the lymphoid structures of the lower part of the small intestine best seen in the Peyer's patches of the ileum just above the ileo-cecal valve. The mesenteric glands and spleen are hyperæmic. Parenchymatous degenerations more or less severe may be found in other organs. The characteristic histological feature is the crowding of the lymph spaces by proliferated endothelial cells.

Perforation and hemorrhage of the bowel, peritonitis, myocarditis, thrombosis, etc., render typhoid fever a dangerous disease. The fatality varies considerably; at one time estimated at 25 per cent, it has been brought down to 10 to 15 per cent by modern methods of treatment and has been given in Minnesota as low as 4 per cent.

Eberth found the organism in 1880 by the examination of the mesenteric glands and spleen of fatal cases. Gaffky cultivated it in 1884. The causal relationship has been a matter of gradual acceptance through evidence furnished by the study of such immunity processes as agglutination, bacteriolysis, and complement deviation, and finally by the high percentage of positive blood cultures. Conclusive evidence is afforded by the development of typhoid fever following the ingestion of pure cultures with suicidal intent.

The agglutination reaction of Gruber and Widal is universally employed in diagnostic laboratories. The blood serum of typhoid

patients, after a certain period of the disease, will cause a characteristi clumping of the bacilli when mixed with pure cultures. The fres serum from a clot may be used, or, more conveniently, dried bloo from which a watery extract can be made. In positive cases the rea tion is present in at least the one-fiftieth dilution and usually in the on hundredth or higher dilutions. The culture employed should b eighteen to twenty-four hours old; it should be freely agglutinable an show no artificial clumping, characters not possessed by all strain especially those recently isolated. Cultures killed by a small pe centage of carbolic acid have been recommended for constancy in plac of the living organisms. When the microscopic method is used th reaction should be distinct in about one hour.

An immune body capable of binding complement in the presen of typhoid antigen is said to occur in typhoid sera before the agglutina tive property appears.

The detection of the typhoid bacillus in the circulating blood ha recently been very widely successful and furnishes the best support f the diagnosis of the disease. While blood culture may be hardl practical in public health laboratories, it has become a routine measu in the modern hospital. Blood is taken aseptically from a vein an about 1 to 5 c.c. is introduced into culture media, of which fluid medi containing ox-bile and agar plating media containing glucose have bee most strongly recommended. The fluid media are used in 100 to 5c c.c. amounts, which serves to dilute the antibacterial properties of th blood while the bile acts as an anticoagulant and possibly also as a antibactericidal measure. Plating lessens the diffusion of the ant bacterial properties and thus favors growth.

The urine and fæces have sometimes to be examined for the presen of *B. typhosus*. It then becomes necessary to differentiate the coloni of this bacillus from those of the colon group. For this purpose man special media have been devised, some depending on the motility of th typhoid bacillus to form a different shaped colony in suitable so media, others based on the fact that some substances such as fuchsi crystal violet, malachite green, etc., inhibit the growth of associate organisms while permitting the typhoid bacillus to develop more or le luxuriantly.

As found in pure cultures, the bacillus is about 1μ to 3.5μ in length and 0.5μ 0.8μ in width (Fig. 167). Filaments are sometimes found several times the leng

the single organism. It is quite regular in shape, straight with rounded ends. ıe bacilli usually occur singly; occasionally two may be attached end to end for a ort time. There are ten to fourteen comparatively stout flagella about two or ree times the length of the organism peritrichic in their arrangement. There e no capsules and no spores. They stain with all aniline dyes, and not infre- ently exhibit more deeply staining areas at the poles. They are Gram-negative. ological and biochemical characters. The minimum temperature is about 10° e optimum 37°, maximum 40 to 41°. It is aerobic and facultatively anaerobic. ıe slight preference for oxygen is probably of little account when such sugars as ıcose are present. The bacillus is not very sensitive to the reaction of media and ll grow in the presence of either slightly alkaline or acid reaction. Alkaline

FIG. 167.—Bacillus of typhoid fever. × 1000. (*After Williams.*)

bstances are produced from peptone. Acid is formed from dextrose, levulose, lactose, mannit, maltose, and dextrin. Lactose and saccharose remain un- anged. Gas is never formed. It is the rule that the *Bacillus typhosus* does not rm indol; certain strains, however, form a trace. The toxins of the bacillus have en very widely studied and several different opinions are held with regard to eir nature. Most evidence supports the idea that the poisons are only set free the destruction of the bacterial bodies. This may be brought about experi- ntally by various means such as the use of lytic or bactericidal sera, by the integration occurring in old cultures, by extraction under great pressures, by turating after freezing in liquid air and by emulsifying cultures, sterilizing by at, then extracting with salt solution. These endotoxins, however obtained out- le of the host, have been found to produce by injection into animals only lytic

and bactericidal sera and not an antitoxin. More recently, however, some c servers claim to have shown in comparatively young cultures the presence of a su stance which upon injection into animals yields an antitoxin and thus comports its after the manner of a true diffusible or soluble toxin. Agar streak cultures show abundant filiform whitish or bluish-gray translucent growth with no special ch acteristics. Broth is uniformly and moderately clouded and only occasionally delicate pellicle may develop. Gelatin colonies are bluish white in color, tra parent and with somewhat notched margins. Stab cultures show more growth the surface, while in the depth the growth is filiform and less abundant. T medium is not liquefied. Milk is not coagulated. In litmus milk there may b trace of acid formed at first, followed by a return to neutral or very slightly alkal reaction. Potato was at one time considered a very valuable differential mediu The growth of the bacillus upon it is quite abundant, glistening, but invisit when the potato is acid. A more alkaline reaction allows a rather heavy yellow growth indistinguishable from *B. coli*. Special media are used in the cultivati of the typhoid bacillus, chiefly for differential purposes. The cultural features these do not show sufficiently striking characters to make it worth while to revi the many that have been devised. Specific agglutinating and bacteriolytic s as well as the complement binding reaction are valuable aids in identifying t bacillus. Resistance to heat and light is not different from that of the average n spore-bearing species. Its thermal death-point is about 56° for ten minutes, for one minute. Exceptionally resistant forms have been found alive in ice af three months. Sometimes the bacilli will remain viable for a month after dryi At other times they die out rapidly. They have been found to be viable for t days in distilled water, while pure sodium chloride dissolved exerted an unfavora influence. In fæces the length of life is from a few hours to several days, or even high as five months in winter. Their life in privies and cesspools is ordinar brief but has been found to extend for thirty days. Of the non-spore-form the bacillus appears to be rather more resistant than the average but succu within five minutes to 1 : 5000 mercuric chloride or 5 per cent phenol.

The organism enters the body through the mouth by means infected fingers, food, milk, and water, etc.

On reaching the intestine the organism probably propagates to so extent before penetrating the intestinal mucosa. It enters into t blood stream and is disseminated throughout the body. Accordi to the endotoxin theory it must slowly be dissolved by the lytic su stances which have been gradually accumulating in response to t primary intoxication.

The organisms have been cultivated from the rose spots and ha been found in vomit without the presence of blood, and in sputu Typhoid meningitis and osteitis occur occasionally. At autopsy t spleen and gall-bladder yield the highest number of positive cultur

is of interest to note too that while the highest percentage (89–90 r cent) of positive blood cultures occurs in the first week and that the rcentage diminishes from then on, the number of positive findings the fæces, on the other hand, runs in the opposite direction.

Generally speaking, one attack confers immunity. Upon what tibodies immunity and recovery depend is a matter of controversy.

The elimination of the bacilli from the body will largely depend upon e stage of the disease, since the blood, especially early in the illness, actically always contains the specific organism; epistaxis is not an important feature as a possible means of disseminating the germs. he bacilli can also escape in the fæces, urine, sputum, and vomit.

In the control of this disease the best place to begin is at the bedside. sinfection of all excreta and of everything which comes into con- t with the patient should be rigorously carried out and in the case of e fæces and urine should ideally be continued until examination can l made showing absence of the organism. It has been estimated that high as 5 per cent of convalescents continue to excrete living typhoid cilli for varying periods from months to years after the disease; the gest time noted has been forty-six years.

The recognition of typhoid carriers will depend absolutely on the ding of the specific germ in the fæces or urine as the case may be. here there are large numbers of suspects, the opsonic index is claimed be an aid in exclusion of the improbable ones, as well as the agglutinin action.

In a general way, prompt recognition of the source of infection such milk, polluted water, bacilli-carriers, etc., together with instruction the individual and the public are often effective in limiting and end- g an epidemic.

While a great many sera have been used therapeutically with some ccess, prophylaxis promises more where it can be widely employed as armies and navies. The artificial immunity is brought about by in- tion of dead cultures. A difference of about 25 per cent has been ted between the percentage of cases in vaccinated and unvaccinated rsons in civil life.

In the United States Army, however, where antityphoid inoculation now compulsory, most remarkably favorable results have been shown ring the year 1913. *Amongst* 90,646 *men, both American and native ops, only three cases of typhoid fever occurred and two of these were*

infected before enlistment; there were no deaths. When comparison made with the best results obtained in the army from sanitary measu alone without the vaccination, Major Russell estimates that in 1913 *th was "only one one-hundred-and-sixty-seventh of the loss of time fr duty because of typhoid fever."*

Asiatic Cholera*

Microspira comma

The disease is endemic in parts of India whence epidemics ha spread throughout the world. America has been visited by seve epidemics and at the sea ports more frequently, chiefly New Orlean

The disease occurs naturally only in man. The incubation peri is from part of a day to ten days, usually about three days.

In its most characteristic form the disease begins with few or prodromata. It is marked by fever, sudden onset of purging a vomiting followed by cramps and severe depression. Evacuatic finally become almost a colorless liquid, "rice-water stools." T cramps may occur in the whole muscular system most frequently the legs and are often extremely painful. A stage of complete colla finally occurs. There are, however, many variations from th typical cases. The mortality is usually given at from 45 to 50 p cent.

After death there are found extensive acute degenerative changes the kidneys; the gastro-intestinal tract shows marked changes in t lining membrane which may be necrotic, sodden and in some pla stripped away.

The cholera vibrios may sometimes be seen in enormous numbers in smears fr typical stools. For a positive diagnosis, however, the organism must be cultivat The usual method is to inoculate a 1 per cent peptone solution from the stool, cubating at 37° for from four to eight hours and sowing plates from the very surf of the liquid, either of gelatin or alkaline agar or both. The vibrios are 3μ to long by about 0.4μ wide, and are curved slightly like a comma or sometimes i half circle. These comma forms are best seen in broth cultures. The e are usually rounded. In young cultures the organisms are usually arran singly, occasionally two may be found end to end in the form of an "S." Ther no capsule, and no spore formation. There is a single terminal flagellum, and

* Prepared by Edward Fidlar.

organism is exceedingly motile. Does not stain as readily with the ordinary aniline dyes as many other bacteria. Fuchsin gives the best result. It is Gram-negative. The optimum temperature for growth is 37° with a minimum of 8° and a maximum of 42°. Plain agar—moist, shining, grayish yellow, and rather thin and transparent as compared with the colon type of colony. A rapid growth takes place in broth, causing a uniform clouding with a more or less well-developed pellicle. In gelatin plates colonies are visible in twenty-four hours and are round, even, and yellowish white, later they become irregular and their surface presents fine refractile granules; within forty-eight hours the colonies are found to be sinking into a small round pit due to liquefaction of the medium (Fig. 169). Concentric rings may appear as liquefaction progresses from day to day. In old cultures the liquefaction assumes a

FIG. 168.—*Microspira comma.* × 1000. (*After Williams.*)

funnel or turnip shape with an air bubble at the surface due to evaporation. Growth in milk occurs without any visible change in the medium. At 37°, on potato, an abundant moist brownish growth. Blood serum is liquefied rapidly. The vibrios prefer the presence of oxygen, yet it is probable that organisms grow under practically anaerobic conditions in the intestine. The reaction of all media must be very distinctly alkaline and even very small amounts of acid are inhibitive. Neither gas nor acid is formed. The production of indol and the formation of nitrites from nitrates occurs regularly. The addition of sulphuric acid is sufficient to give the nitroso-indol reaction, which from its association with this bacterium has been called the cholera red reaction. No pigment is produced. Majority of freshly isolated cultures have hæmolytic powers. It is generally considered that there is only an endotoxin, but it is strongly asserted by some that a soluble toxin is formed. Thermal death-points are 60° for ten minutes, 95° to 100° for one minute. Vibrios are quite sensitive to low temperature and at most have been found viable in ice only after a few days. The vibrios are quite susceptible to the ordinary disinfectants.

The cholera organism gains entrance through the mouth.

Having succeeded in passing the acid secretions of the stomach the vibrios probably develop with great rapidity in the small intestine.

The peculiar conditions favorable to the development of the organism in the intestine are unknown. A previous gastro-intestinal disturbance is probably necessary even though slight.

The organisms have rarely been demonstrated in blood cultures. The gall-bladder gives the highest percentage of positive cultures.

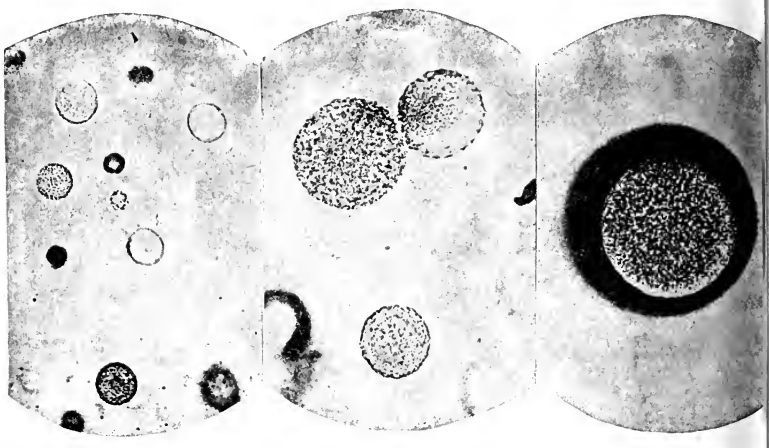


FIG. 169.—Microspira comma. Colonies on gelatin plates. *a*, Twenty-four hours old; *b*, thirty hours old; *c*, forty-eight hours old. (*After Fraenkel and Pfeiffer from Williams*,

Highly lytic and agglutinating sera can be developed experimentally but little or no antitoxic power can be demonstrated.

Protective inoculation has shown considerably more encouraging results than serum therapy.

The cholera vibrios are eliminated in the discharges. Water and uncooked food becoming contaminated with cholera excreta are the chief means by which the epidemic is spread, so that its epidemiology is similar to that of typhoid fever.

DISEASES OF UNKNOWN CAUSE*

Scarlet Fever, Measles, German Measles, Duke's Disease, Smallpox, Chickenpox, Mumps†

These diseases constitute a group the actual biological causes of which are unknown, yet which show analogies to diseases the causes of which are known, so close as to make tenable the hypothesis that they are due to similar causes.

Mumps is in a class by itself, its characteristics, well known to the laity, marking it off from the others sharply. Like the others it is infectious; it is derived only from a preceding case; it has a more or less definite incubation period (*i.e.*, an interval between the date of infection and the first development of symptoms, during which ordinary health is enjoyed), and a prodromal stage (*i.e.*, a period in which fever, headache, and other more or less marked constitutional symptoms exist without any marked characteristic symptom). Then appears the swelling of the parotid salivary glands just in front of the ears with some pain. The symptoms usually amend after a few days and the patient goes on to full recovery. There is no rash nor any great disturbance of the intestinal tract or internal organs as a rule, although metastases, affecting the mammæ, ovaries or testicles develop at times; and secondary complications sometimes are found.

Smallpox and chickenpox together form a group quite often confused clinically, especially in the early stages and especially when smallpox is prevalent in mild form. They have incubation periods, approximating about twelve days, in smallpox varying little from this period, in chickenpox varying widely from it. Smallpox has rather severe prodromes, backache, headache, fever, and sore throat, the rash appearing on the third or fourth day. Chickenpox usually has light or no prodromes, the rash appearing on the same day or within twenty-four hours, as a rule. In both diseases the face, chest, back, arms, hands, legs, and feet are likely to show eruption, but chickenpox tends to show the greatest number of spots "under cover," *i.e.*, on the parts usually covered by clothing, while smallpox tends to show the majority upon the face, neck, arms, wrists, hands, legs and feet rather than on the body. The skin lesions themselves differ very markedly, the typical lesions of chickenpox being superficial, thin walled, high, rounded,

* Arranged alphabetically except group of diseases placed first.

† Prepared by H. W. Hill.

and filled with clear liquid, those of smallpox being deep seated, tense, opaque, with a tough covering of epithelium. There are many other points of distinction, and any one familiar with the two diseases can hardly fall into error when dealing with typical cases at whatever stage they are encountered. To the layman's eye, however, the two are often indistinguishable.

Scarlet fever, measles, German measles, and Duke's disease are often likewise confused by the laity and even by physicians who have not had opportunities for extensive study.

German measles is clinically related to true measles somewhat as chickenpox is to smallpox, *i.e.*, they are wholly distinct diseases yet show characteristics easily confused on superficial consideration. Duke's disease is perhaps not a distinct entity; much has been said on this point and a satisfactory decision will probably never be reached until the causative agents have been found. It may be described briefly for clinical purposes as a variety of German measles having a scarlatiniform instead of a measly rash.

Scarlet fever has an average incubation period of about five days, or perhaps sometimes less. The prodromes are those usual to all these infections—headache, fever, and sore throat, but the latter is especially severe. Within twenty-four hours the rash appears usually on the chest first, a bright scarlet superficial punctate flush, extending rapidly over the body.

In measles the incubation period is longer, averaging nine or ten days, almost without any variation. The prodromes, headache, fever, and sore throat, are accompanied by very marked coryza and photophobia, catarrh and "cold on the chest."

The rash appears about the fourth day, appearing on the face and back but rapidly extending. It is darker, bluer, and deeper than the scarlet fever rash and unlike the latter is palpable. Koplik's spots appear on the buccal membrane early in the disease.

In German measles the prodromes are so indefinite that it is difficult to determine their length; very commonly the rash is the first thing noticed. It appears on the face, chest, back, and arms as a light subcuticular mottling (measles type) or a more uniform pink flush (scarlatiniform or Duke's type); with this rash the eyes show some injection and slight photophobia develops. The attack passes off quickly, without complications.

Canine Distemper*

This disease (Maladie des jeunes chiens; Fr.) is so widespread that the great majority of adult dogs may be regarded as having suffered from an attack and recovered. It is practically confined to very young animals and, so far as known, no species except dogs are susceptible. The disease is attended by more or less extensive coryza with a discharge from the eyes. There is an eruption on the skin and frequently nervous disorders of various kinds. The animal becomes emaciated and may die from bronchial pneumonia. No organism has been fully accepted as the cause of this disease. Carré has reported that he has succeeded in passing the infectious agent in nasal discharges through earthen filters, the filtrate reproducing distemper in characteristic form. Ferry has announced the discovery of an organism as the causal agent. Some attempts have been made to produce a protective serum.

Cattle Plague*

This disease (rinderpest), which is probably the severest and most contagious of all cattle diseases, is characterized by high fever and lesions of the intestinal tract. It does not exist in the United States but is found in Europe, S. Africa and Asia. Extensive outbreaks have occurred in the Philippine Islands. The cause of cattle plague has never been isolated and the indications are that it is caused by an invisible microörganism. Cattle plague was the first disease in which the process of "hyperimmunization" was practised. Immune cattle receive massive injections of blood from diseased cattle. After this treatment the blood serum of the immune is used to protect non-immunes. Enormous quantities of this serum are prepared and applied yearly by the British government in India.

Chicken Pest*

This disease (Hühner Pest; Ger.: Peste aviaire; Fr.) of fowls, which is to be distinguished from chicken cholera, is not known in the United States, but has caused extensive losses of fowls in Europe, particularly in Italy. Affected chickens cease eating, the feathers become ruffled and the comb darker in color. The lesions found at autopsy are not

* Prepared by M. Dorset.

constant, but a pericarditis is usually seen. There may be, also, congestion of the lungs, liver, and kidneys. The intestinal lesions are not as marked as is the case in chicken cholera.

Chicken pest has been shown to be due to an invisible microörganism which is present in the heart blood and in practically all of the organs of the body. Most fowls are susceptible; guinea-pigs and mice are refractory to the disease. The virus passes through Berkefeld and Chamberland F cylinders; it is quite resistant to drying but is destroyed by an exposure of half an hour to a temperature of 60°. Several authorities have passed the filtered virus through four or more hens successively, thus demonstrating positively that the filtered virus is capable of multiplication.

Contagious Bovine Pleuro-pneumonia*

This disease affects cattle only; it is highly infectious and produces an inflammation of the lungs and pleural membranes. Thirty years ago bovine pleuro-pneumonia was quite prevalent in the United States but has since been eradicated through the efforts of the Federal Bureau of Animal Industry in coöperation with State authorities. It still exists in European countries.

The microörganism of bovine pleuro-pneumonia is generally classed among the invisible viruses, though unlike the other organisms of this class it has been cultivated artificially and is just visible at a magnification of 2,000 diameters. The artificial cultivation of this virus was accomplished by Roux and Nocard through the use of the very ingenious "collodion sac method." A small amount of virus from a diseased cow was placed within a small thin-walled sac of collodion; after being hermetically sealed the sac was placed in the peritoneal cavity of a rabbit where it remained for several weeks. At the end of this time the unbroken sac was removed and the previously clear fluid within was found to be slightly opalescent. Microscopic examination revealed numberless minute motile bodies so small, however, that their exact form could not be determined. Later the organism was successfully cultivated outside of the animal body in a specially prepared bouillon. These cultures produced the disease when inoculated into susceptible cattle. When the virus is diluted it will pass through the

* Prepared by M. Dorset.

Berkefeld and Chamberland F cylinders, but not through the Chamberland B cylinder.

Cowpox, Horsepox, and Sheeppox*

Variola refers to a condition or disease in man and animals, characterized by fever and the appearance of skin eruptions which successively assume the form of papules, vesicles and pustules. The disease is frequently found in the human species (smallpox), cattle (*variola vaccinia*, cowpox), horses (*variola equinæ*, horsepox) and sheep (*variola ovina*, sheeppox). It is possible that some other species may be susceptible.

On account of the fact that vaccination of man with virus from cases of cowpox affords remarkable protection against smallpox, it appears reasonable to believe that cowpox virus or smallpox vaccine is a modified form of smallpox virus. This fact, together with the occasional positive results of various experiments in which other species of animals have at times evidenced susceptibility to cowpox virus, strongly suggests the possible etiological relationship of the diseases in different species to each other and to smallpox in man. However, conclusive proof supporting this suggested relationship does not exist. The specific causative factor of smallpox or of cowpox is not known.

Cowpox is a very common disease, perhaps having been prevalent in England and Europe for centuries. Its presence has frequently been observed in various countries since 1796 when Jenner contributed to the world his important discovery relative to smallpox vaccination.

Many attempts have been made to isolate the causative factor of cowpox. Early investigators frequently secured mixed and pure cultures of various organisms, including different species of micrococci, streptococci and bacilli from vaccine lymph. None of these organisms were peculiar to the virus, and at present there exists no definite evidence that the infectious agent of vaccine lymph is of bacterial nature. Pfeiffer, Guanieri, Plimmer, Councilman, MacGrath, Brinckerhoff and others, after observing the presence of apparent cellular elements, or relatively large flattened bodies in vaccine lymph, have suggested the possible protozoan nature of the causative agent. Attempts have been made, with more or less success, to cultivate these

* Prepared by W. E. King.

bodies in collodion capsules in the peritoneal cavities of experimental animals. According to some investigators the virus has been passed through a Chamberland filter. The failure to discover the causative factor, according to the present methods, may be due to the inability of microbiologists to cultivate or stain the specific agent.

Cowpox is characterized by eruptions which usually occur on the skin of the teats and udder. The material contained in these pustules is transferred to other animals by the hands of the milker and through other possible means of dissemination. The chief channel of infection appears to be through an abrasion in the skin. The period of incubation of cowpox is about two days. The virus possesses relatively weak resistance to heat, light and chemicals. The control of the disease depends chiefly upon precautions relative to the transmission of the virus on the hands of the milker from infected to healthy cows.

Horsepox may be diagnosed by the appearance of the characteristic pustules usually upon the skin, nasal mucosa and buccal membrane.

Sheeppox is characterized by the presence of the typical skin eruptions, following a rise of temperature.

Dengue*

This disease (break-bone fever) of man occurs in all parts of the world. It is characterized by a sudden attack, intense prostration and severe pains in the muscles and joints. The fever during the attack shows a characteristic curve. There is a sudden rise of and maintained temperature for several days. Then a remission and a second rise of temperature which is less than the first.

Our knowledge of the cause of this disease rests chiefly upon researches of Ashburn and Craig (The Journ. of Infectious Diseases, Vol. X, p. 440, 1907). These authors conclude that dengue is not contagious in the ordinary sense but that it is transmitted through the bite of the mosquito (*Culex fatigans*). No visible organism could be demonstrated in either fresh or stained specimens of blood from patients affected with dengue although such blood was capable of producing a typical attack of dehgue when inoculated intravenously into healthy men. The authors likewise show that blood from a case of dengue retained its infectiveness after passage through a filter made of diatomaceous earth. The organism of dengue fever is therefore probably of ultramicroscopic size.

* Prepared by M. Dorset.

Foot-and-mouth Disease*

Foot-and-mouth disease is primarily a disease of cattle, though the other domestic animals and man may be attacked. The disease is very contagious and is characterized by the eruption of vesicles in the mouths, on the udders and on the skin surrounding the hoofs of cattle. It is very prevalent in European countries. There have been two outbreaks in the United States both of which were promptly eradicated by vigorous repressive measures instituted by the Federal authorities.

The cause of this disease is an invisible microörganism which exists in the lymph from the vesicles which form in the mouths and on the feet of cattle. This virus has never been cultivated artificially. It passes through the Berkefeld cylinder but not through the finer-pored Kitasato filters; it is quickly destroyed by formaldehyde, carbolic acid and similar disinfectants.

The disease is readily transmitted from one animal to another by contact and the contagion may persist for some time in the manure, or straw from infected stables. The milk of infected cows has been said to produce the disease in children.

Animals which recover from an attack remain immune for a short time only; it is therefore not surprising that no satisfactory means of artificial immunization has been devised.

Hog Cholera*

The first recorded outbreak of hog cholera in the United States occurred in Ohio in the year 1833 and it now exists in practically all sections of this country. Hog cholera is most prevalent in the late summer and fall, although outbreaks are reported at all seasons of the year. All races of hogs are susceptible and the average mortality is about 80 per cent. In the United States alone the losses from hog cholera are estimated to average at least $15,000,000 annually. Hogs only are attacked. This disease is supposed to have been introduced into the United States through the importation of hogs from Europe, where it is known under the names "swine fever" (Br.), "schweinepest" (Ger.), and "peste du porc" (Fr.).

* Prepared by M. Dorset.

The essential features of hog cholera may be briefly summarized as follows: Extreme contagiousness. Symptoms of severe illness accompanied by fever, loss of appetite, weakness and diarrhœa. Hæmorrhagic lesions in the various organs and lymphatic glands and round button-like ulcers in the large intestine. Immunity in hogs which recover.

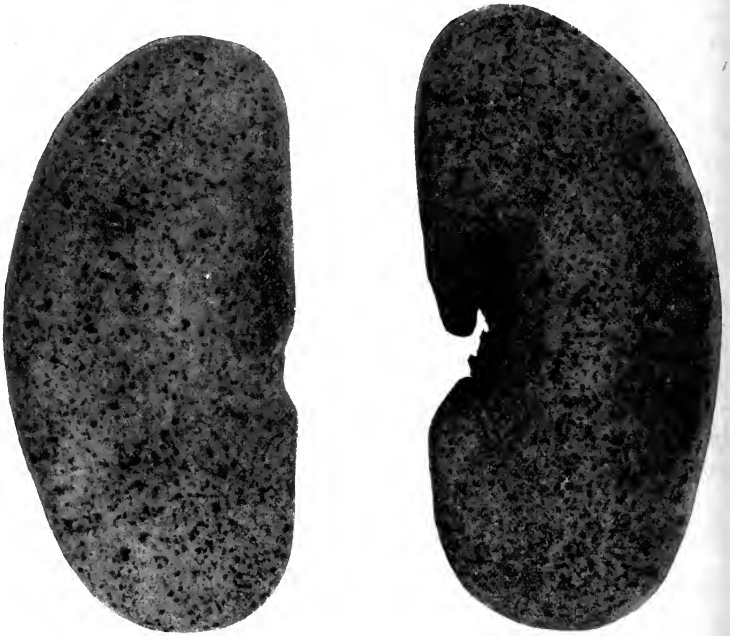


FIG. 170.—Hemorrhagic points on kidneys of hog-cholera hog. (Original.)

The etiology of hog cholera has long been the subject of scientific controversy, but it is now generally acknowledged that the cause of this disease is an invisible microörganism which exists in the blood, the internal organs, and the urine of infected hogs. The fact that this disease is caused by an invisible microörganism was demonstrated as follows:*

The blood serum of hogs infected with hog cholera acquired in the

* Bulletin 72, Bureau of Animal Industry, U. S. Dept. Agriculture, 1905.

atural way is very infectious for non-immune hogs, the disease being eadily transmitted by the subcutaneous injection of small amounts. he disease produced by this subcutaneous injection is identical in all espects with the disease as it occurs in nature. If, now, this infectious erum is diluted with normal salt solution or with ordinary bouillon 1 to 10) and passed through either a Berkefeld or Chamberland lter, the filtrate, though free from all visible microörganisms, still re- ains the power to produce hog cholera by subcutaneous injection. he disease which is produced in this manner by the filtered hog holera serum is identical in all respects with the disease produced by he unfiltered serum and also with the disease as it occurs in nature. he hogs which receive the filtered serum present the symptoms and sions of hog cholera. The disease set up in this manner is very ontagious and hogs which recover from the inoculation of filtered erum are thereafter immune against hog cholera. By repeated noculation and filtration this virus may serve to infect successively a rge number of hogs.

The invisible virus of hog cholera, in view of its ability to pass rough the Chamberland B filter, must be regarded as one of the nallest of the invisible microörganisms. It has never been cultivated rtificially, hence, aside from its disease-producing qualities, we have ttle knowledge concerning it. We do know, however, that the virus quite resistant to such common disinfectants as carbolic acid and ichloride of mercury and that it is quickly destroyed by a 3 per cent olution of *liquor cresolis compositus* (U. S. P.) as well as by a 5 per nt solution of antiformin. When preserved in sealed glass bulbs a cool dark place, the virus retains its activity for six months or nger. Rabbits, guinea-pigs, and other small animals are entirely susceptible to inoculations of the filtered virus in amounts which ould prove fatal to hogs.

The virus of hog cholera is known to be thrown off from the body rough the urine, and it is probably also eliminated through the feces. herefore any agency which would serve to carry a particle of dirt from fected hog yards might be the means of disseminating the virus. As any sick hogs find their way to the public stock yards through ship- ent by rail, all stock cars and stock yards are to be regarded as rmanently infected. It appears to be impracticable to prevent the read of the disease by methods of quarantine and disinfection,

owing to the impossibility of enforcing such measures thoroughly. has recently been found that a protective serum against hog chole may be produced by "hyperimmunization." The process consists giving immune hogs large doses of blood taken from hogs sick of h cholera. As a result of this blood treatment their serum acquires t power to protect non-immunes. Injections of serum from hyp immunized animals confers a passive immunity, while the simultaneo injection of serum with a small amount of virus produces an acti immunity.

Bacillus Choleræ Suis (*B. suipestifer*).—No description of t etiology of hog cholera would be complete without a reference to th bacterium which was long regarded as the cause of hog cholera. It found after death in the blood and organs of the majority of ho affected with hog cholera and in this rôle of secondary invader it doubt tends to increase the mortality from the disease. *B. chole suis* is a small, very actively motile, non-spore-bearing bacillus wi rounded ends, which stain readily with the ordinary aniline dyes. does not stain by Gram's method. This organism is easily cultivat on the ordinary media; gelatin is not liquefied; milk is not coagulat but acquires an acid reaction at first; this changes after a week or mo to an alkaline reaction. Gas is produced in bouillon containi dextrose, but lactose and saccharose are not affected. Rabbits a guinea pigs succumb within four to ten days to small doses of th organism. Hogs are much more refractory. It is only after the a ministration of large doses that they show any symptoms of illne following subcutaneous injections. By feeding pure cultures of *choleræ suis* or by injecting these intravenously a considerable numb of hogs will succumb and at autopsy present lesions which correspo quite closely to those seen in naturally acquired cases of hog chole There are, however, certain important differences between the disea produced by *B. choleræ suis* and the natural disease hog cholera. F example, hogs infected with *B. choleræ suis* do not transmit the disea to other hogs by contact. The blood of hogs infected with *B. chole suis* does not produce disease when injected subcutaneously in other hogs, and, in addition, hogs which recover from illness produc by injections or feedings of pure cultures of *B. choleræ suis* have immunity against the natural disease hog cholera.

Horse Sickness*

This disease affects the equine species only and appears to be confined to South Africa. It is most prevalent in summer and appears to be transmitted by the bite of an insect, as it is not contagious but may be communicated to susceptible horses through blood inoculations. This disease manifests itself by producing severe inflammatory changes in the lungs and in the tissues of the head and neck and is attended by a high mortality. No visible organism has been found which will produce horse sickness and as McFadyean and Nocard have shown that the virus is capable of passing through the finest bacteria-proof filters, this disease is probably caused by an invisible microörganism. Blood containing the microörganisms of horse sickness may be kept in sealed bulbs in the dark at room temperature for more than two years without losing its infectiveness. The virus is quite resistant to drying and may survive heating for ten minutes at a temperature of 75°.

Infantile Paralysis*

As indicated by its name, this disease (epidemic poliomyelitis) is usually seen in children. It has long been known to exist in both Europe and America, occurring generally in sporadic form. During the last decade, however, its prevalence has greatly increased and a number of well-defined epidemics have been reported. Though the character of this malady long ago led to the belief that it was caused by a microörganism, this fact was not definitely proven until the year 1909 when Landsteiner and Popper in Germany, and Straus and Huntoon and Flexner and Lewis in the United States, succeeded in transmitting the infection to monkeys. So far as is now known, none of the lower animals except monkeys are susceptible.

The symptoms and effects of infantile paralysis are extremely variable. Paralysis is by no means constant, many cases being very mild and thus possibly escaping detection. In the severer forms of the disease paralyses of various types and degrees are seen. When recovery takes place the paralysis may appear to improve only to be followed by atrophy of certain groups of muscles, resulting in deformity and permanent lameness. These effects are caused by the destruction of certain nerve centers in the spinal cord.

* Prepared by M. Dorset.

As stated above, the microbial origin of infantile paralysis was firs demonstrated by the inoculation of monkeys, Flexner and Lewis havin successfully carried the infection through a long series of monkeys b successive intracranial injections of an emulsion of the spinal cor taken from infected animals. The microörganism passes through th Chamberland and Berkefeld filters with little or no loss in diseas producing power. "Flexner and Noguchi, employing the techni previously used for cultivating pathogenic spirochætes, have succeede in obtaining from infected tissues cultures of a minute round organis which they believe to be the cause of infantile paralysis." The viru withstands freezing or drying for long periods of time but is quickl destroyed by heating at a temperature of 50°. It is likewise quickl killed by the ordinary disinfectants. Monkeys may be infected by th subcutaneous, intraperitoneal, intravenous, or intracranial injectio of material from an infected spinal cord, but attempts at infectio through feeding have been unsuccessful. The virus appears to b eliminated from the body through the nasal mucous membranes.

It appears probable that one attack of the disease protects from second attack. No cases of a second attack have been reporte Furthermore, monkeys which have recovered from the infection appe to be entirely immune as shown by Flexner. Active immunity i monkeys has been established by repeated infections of graduall increased amounts of the virus. The blood of human beings and monkeys that have recovered from an attack of the disease is capab of neutralizing a certain amount of the virus. This protective qualit of the blood serum may be increased by repeated inoculations of viru and infection in monkeys can be prevented by injecting simultaneousl the virus into the brain and the serum into the sub-arachnoid spac The serum treatment of this disease is, however, not developed to suc a state that it can be regarded as of practical use.

Louping Ill—Trembling in Sheep*

This disease is known only in Scotland and is essentially a disease sheep. It is characterized by a variety of nervous phenomena, such a trembling, irritability, and convulsive movements, which are followe later by partial or complete paralysis. The chief lesions are found i

* Prepared by M. Dorset.

he meningeal membranes. This disease is supposed to be transmitted by ticks, not the wingless fly which is generally called a "sheep tick" in the United States, but true ticks, belonging to the genus *Ixodes*. The specific microörganism has not been discovered.

Pellagra*

Pellagra is a disease of man characterized by the annually recurring manifestation, each spring or summer, of erythema on the backs of the hands and forearms and sometimes on the face and neck, feet and ankles, coupled with digestive disorder and more or less well-marked mental disturbances. During the winter the signs of the disease usually disappear.

At present there are two main groups of theories concerning the causation of pellagra, each of which includes a multitude of hypotheses. According to one group of theories pellagra is a food poisoning due to eating maize (Indian corn); according to the other, pellagra is a specific infectious disease not necessarily associated with the ingestion of corn. None of the theories concerning causation is supported by conclusive evidence. The evidence against the corn theory marshalled by Sambon† and others‡ has greatly weakened the almost general belief in this theory which formerly obtained. Some prominent zeists§ have recently shown a tendency to ascribe pellagra not essentially to the use of maize but to a supposed deficiency or lack of a necessary something in the diet. This change of opinion has been caused in part by the failure of the maize theory when put to the test of actual observation and in part by an eager application to pellagra of the facts learned in the study of another disease, namely, beriberi. To the writer it seems very improbable that this new phase of the dietary theory will survive as long as has the maize theory proper, although it has recently received enthusiastic support from the U. S. Public Health Service.‖

* Prepared by W. J. MacNeal.

† Sambon, Brit. Med. Journ., Nov. 11, 1905; Journ. Trop. Med. and Hyg., 1910, Vol. XIII pp. 281–282; 287–300; 305–315; 319–321.

‡ Siler and Garrison, Amer. Journ. Med. Sciences, July, 1913, Vol. CXLVI, p. 42; *ibid.*, Aug., 1913, Vol. CXLVI, p. 238; Siler, Garrison and MacNeal, Journ. A.M.A., 1914, Vol. LXII, pp. 8–12.

§ Sandwith, Trans. Soc. Trop. Med. and Hyg., 1913, VI, p.p. 143–148; Weiss, Riv. Pellagrologica Italiana, 1913, XIII, 90; Driscoll, Southern Med. Journ., 1913, VI, pp. 400–401.

‖ Goldberger, Joseph, and collaborators, Public Health Rep., June 26, 1914, Vol. XXIX, p. 1683; *ibid.*, Sep. 11, 1914, Vol. XXIX, p. 2354; *ibid.*, Oct. 22, 1915, Vol. XXX, p. 3117; *ibid.*, Nov. 12, 1915, Vol. XXX, p. 3336.

According to the second theory, pellagra is a specific infectious disease, in which poor nutrition is one of the important predisposing factors. The epidemiological study* of pellagra, as it has developed and spread in certain parts of the southern United States, has brought to light evidence of its infectious nature which, to the writer, seems very convincing. The same investigation has also strongly suggested that the infection is intestinal and transmitted in much the same way as in typhoid fever. A specific microbic cause of pellagra has not been identified.

Rabies†

Lyssa or Rabies, the madness of dogs, was recognized as a definite disease of animals and man by the peoples of ancient times. The disease is generally distributed throughout the civilized world except in those places where special measures to stamp it out have been enforced. It does not arise spontaneously but is an infectious disease transmitted from animal to animal. Rabies is primarily a disease of wolves and dogs, and the bite of a mad dog is the most frequent cause of the disease in other animals and in man. It is not uncommon in horses and cattle, and all mammals appear to be susceptible to it.

In animals inoculated by injection of the most virulent virus (fixed virus) directly into the brain, the symptoms of rabies appear in four to six days and death usually occurs on the seventh day. Accidental inoculation by the bite of a rabid animal (street virus) rarely causes the symptoms to appear before three weeks, and the onset may be delayed for six months or a year. Not all persons or animals bitten by rabid animals take the disease; probably not more than one in four or five. This variability depends upon several factors, the most important ones being the virulence and the amount of disease virus, and the part of the body into which it is introduced. Bites upon the face or hands, because of the rich *nerve supply* of these regions and the lack of protection by clothing, are likely to result in rabies sooner than bites elsewhere.

After the disease has developed, death is inevitable. In all animals the symptoms are those of a nervous disorder. At first there is excita-

* Siler, Garrison and MacNeal, Arch. Int. Med., 1914. Vol. XIV, pp. 292–373; *ibid.*, 1914 Vol. XIV, pp. 453–474; Journ. A.M.A., 1914, Vol. LXIII, pp. 1090–1093.

† Prepared by W. J. MacNeal.

on, and this is followed by paralysis and death, the relative length the two stages varying in different animals. In the dog the disease ns its course in six to eight days. It begins with altered behavior of e animal, itching of the infecting scar, changed appetite, and slight ver. The dog swallows grass, stones, and pieces of wood. As the age of excitement becomes more fully developed, the animal may n away and may travel fifty miles or more, snapping and biting from ne to time, as the fits seize him, everything in his path. Finally the citement is succeeded by paralysis, beginning in the lower jaw, which ngs down. Then the hind legs fail, and soon the dog, no longer able drag himself along, lies completely paralyzed, greatly emaciated, d soon dies. In the rabbit the stage of excitement is hardly notice-le, but the animal passes quickly into the paralytic stage, dying after o or three days. This type of paralytic rabies sometimes occurs in gs, but is more commonly observed in herbivorous animals.

In man there is a first psychical change, irritation in the scar of the fecting wound and rise of temperature. The first diagnostic symp-m is usually a sudden spasm of the pharynx upon an attempt to allow water. This convulsive seizure is repeated upon every at-mpt to drink, and soon even the sight of water or the thought of it ings on the attack. The cramps extend to other muscles of the dy, and the patient may die in a convulsive seizure, or may pass into e succeeding paralytic stage and die peacefully. The dread of water hich is often so prominent a symptom in man has given the name of drophobia to the disease. Consciousness and general intelligence e not particularly affected. The duration of active symptoms of the sease is from three to six days.

Rabies can be transmitted with certainty by injecting a small mount of emulsified spinal cord of the rabid animal into the brain of rabbit or guinea-pig. Inoculation under the skin is not quite so rtain, and inoculation into the blood stream, or by feeding, generally ls to transmit the disease. When first removed from a rabid dog, e virus (street virus) kills rabbits in from two to four weeks, but after peated transfer from rabbit to rabbit in series, the period of incuba-n is shortened until death occurs quite regularly in six or seven days er inoculation. Beyond this there is no further increase in virulence rabbits, and this six- or seven-day virus is called the "fixed virus."

The localization of virus in the body of the rabid animal has been

worked out by experimental inoculations. The central nervous syste is always virulent, as are also the salivary glands and the saliva. Tl peripheral nerves frequently contain the virus, less commonly oth glands and secretions such as the tears, urine and milk. The virus h never been found in the liver or spleen, or in the blood. Under ordina conditions, the chief source of danger is the saliva of the rabid anim especially when this is introduced into a wound.

Rabies may be recognized in a dog in one of the three ways: observ tion of the course of the disease; autopsy; inoculation of test-anima and observation of the course of the disease in them. If the suspect dog is chained or caged, the question of rabies may be settled in a fe days, for, if mad, the raging stage will be succeeded by the characte istic paralysis and death. If the dog has already been killed, a caref autopsy may show the absence of normal food from the digesti tract and the presence there of abnormal ingested material, high suggestive of rabies. Microscopic examination of the central nervo system is, in the hands of an expert, a reliable method of diagnos which in this case depends upon the finding of the characteristic Neg bodies in the specimen. For confirmation of the diagnosis, a porti of the brain or spinal cord, removed without contamination, should injected into the brain of test animals, and the effects observed. Th last test carried out by experienced observers is justly regarded as t most trustworthy of all.

THE NEGRI BODIES.—The peculiar bodies found by Negri the central nervous system of rabid animals seem to occur invariab and exclusively in this disease, and it is probable that they represe stages in the development of the infectious agent. These bodies a especially numerous and most easily demonstrated in the Ammor horn of the brain in cases of the natural disease in dogs (street rabie Excellent results may be obtained by the method of Lentz.*

Transverse sections, 2 to 3 mm. in thickness, of the Ammon's horn of the s pected brain are hardened in acetone at 37° for one hour, then transferred melted paraffin (melting point 55°) in the paraffin oven at 58° for one and one-h hours and embedded. Sections, 2 to 3μ in thickness, are then cut with the mic tome, floated upon lukewarm water and mounted upon perfectly clean flam glass slides. The excess of water is carefully removed with filter paper and t slides are then completely dried on a warm plate at 45° or in the incubator

* Lentz, Otto, Ein Beitrag zur Faerbung der Negrischen Koerperchen, Centralbl. f. B etc., I Abt., Bd. XLIV, pp. 374–378.

37°. The sections adhere perfectly as a rule and are dry enough to proceed with after ten to fifteen minutes. The slides are next transferred to xylol to remove the paraffin and thence to absolute alcohol. The staining procedure is as follows:

1. One minute in alcoholic eosin.
   Eosin extra B-Hoechst, 0.5.
   Alcohol, 60 per cent, 100.0.
2. Wash in water.
3. One minute in Loeffler's methylene blue.
   Saturated alcoholic solution of methylene blue, B-Patent Hoechst, 30.0.
   Potassium hydroxide solution, 0.01 per cent 100.0.
4. Wash in water.
5. One minute in Gram's solution.
   Iodine, 1.0.
   Potassium iodide, 2.0.
   Distilled water, 300.0.
6. Wash in water.
7. Methylic alcohol until the preparation becomes entirely red.
8. Wash in water.
9. Loeffler's methylene blue again for thirty seconds.
10. Wash in water.
11. Dry carefully by pressing with filter paper upon the preparation.
12. Differentiate in alkaline alcohol until only a weak eosin color remains in the preparation.
   Absolute alcohol, 30.0 c.c.
   Sodium hydroxide, 1 per cent solution in absolute alcohol, 5 drops.
13. Differentiate in acid alcohol until the collections of ganglion cells in the gray matter are still faintly blue while the rest of the section is free from blue (macroscopic).
   Absolute alcohol, 30.0 c.c.
   Acetic acid, 50 per cent, 1 drop.
14. Wash quickly in absolute alcohol.
15. Xylol.
16. Balsam and cover-glass.

Steps 5 to 9, inclusive, may be omitted to save time at some sacrifice in the final result. The Negri body is stained pink with blue granules in its interior. The nerve cells are stained pale blue.

Although sections are most satisfactory for diagnostic purposes and especially to show the relation of the Negri bodies to the ganglion cells, it is usually possible to recognize the Negri bodies in smears, after a little experinece. For this purpose a portion of the gray matter of the Ammon's horn is crushed by gentle pressure between two perfectly clean flamed slides and spread upon them by carefully slipping the slides apart. The moist smears are at once fixed in methyl alcohol for one minute, then washed in absolute ethyl alcohol, whereupon they are ready to be stained by the procedure outlined above.

The Negri bodies (Fig. 171) appear as round or somewhat triangular structures, for the most part inside the ganglion cells. Their size varies considerably, from 1μ to 27μ in diameter, the majority measuring about 5μ. In the interior of the Negri body, smaller structures of variable size and number can be seen. These granules

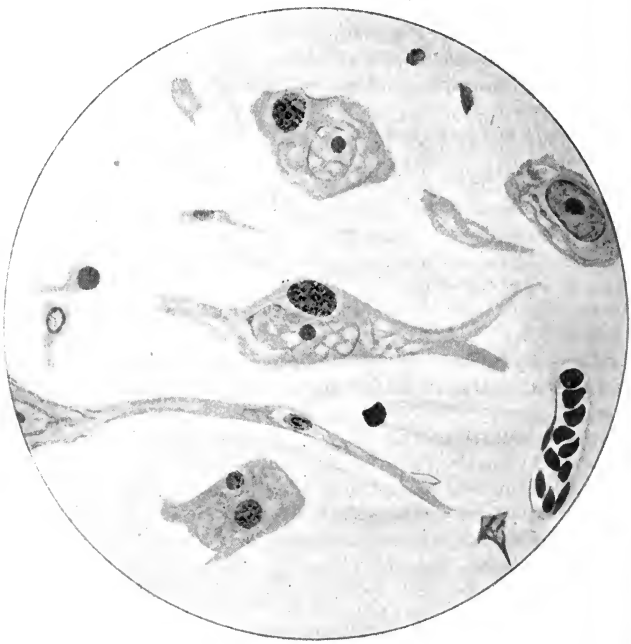


FIG. 171.—Section through the *cornu ammonis* of brain of a rabid dog; stained by the method of Lentz. Five Negri bodies of different sizes are shown, enclosed within the ganglion cells. The smallest contains only three minute granules. (*After Lentz, Centralbl. f. Bakt*, 1907, Abt. I, Vol. XLIV, p. 378.)

may be differentially stained as in the Lentz method. Some careful students of rabies regard the Negri bodies as protozoa and consider them to be the infectious agent. Proof of this belief is still lacking inasmuch as it has not yet been conclusively shown that they are actually living organisms.

A wound infected by a rabid animal should be thoroughly cauterized, under anæsthesia if desired, at the earliest possible moment, and

his cauterization should not be omitted even if twenty-four hours have lapsed. Cauterization cannot be relied upon to prevent the development of rabies, but it does serve to prolong the incubation period. The Pasteur treatment should then be instituted as soon as possible, and it has proved to be practically an absolute preventive, provided the incubation period of the disease is sufficiently prolonged for the treatment to become effective, and this is usually the case. The treatment consists in the daily subcutaneous injections of altered fixed virus for a period of about three weeks, and is most effectively given at Pasteur institutes devoted especially to this work. Valuable animals as well as man may be successfully treated in this way.

The general prevention of rabies depends almost solely upon the efficient control of all dogs in a community. General muzzling, strictly enforced, is a certain preventive of rabies, and in countries where this is done rabies is practically unknown.

Swamp Fever*

This is a comparatively new disease of horses so far as definite information is concerned, but is in reality an old disease that has been described under a variety of names for many years. It is known by various names as infectious anæmia, malarial fever, horse typhoid, "plains" paralysis, and pernicious anæmia, and has been recognized in many portions of the Western United States and Canada.

This disease is usually of chronic type, but acute cases have been reported. There is usually a long illness extending from a month to a year or more, and marked by periods of fever and debility, alternating with periods of apparent recovery. The phase of apparent illness is characterized by mild fever, general weakness, and staggering gait, and the disease terminates fatally, as a rule. The peculiar features of the disease are the alternating periods of illness and recovery, unthriftiness in spite of unusually good appetite, pallor of mucous membranes, dropsical swellings of the belly and limbs.

It has been satisfactorily proved that swamp fever is caused by filterable virus present in blood, urine, and fæces.

Under artificial inoculation with blood, the period of incubation varies from ten to forty days. The natural method of infection is

* Prepared by M. H. Reynolds.

unknown, but there are reasons for believing that infection does not easily occur by way of the digestive organs nor through the respiratory organs. The disease is apparently not communicated by simply stabling diseased animals with healthy animals.

Distribution in the body is very general, as shown by the wide distribution of characteristic lesions, and as shown by the fact that the blood is infectious.

The virus which causes swamp fever reduces greatly the number of red blood corpuscles and also produces local hæmorrhages which are most frequently small and sharply defined. The reduction of red blood cells produces marked pallor, and there gradually develops noticeable emaciation.

Post-mortem lesions in many cases are slight. The hæmorrhages may involve subcutaneous and intermuscular tissues, spleen, and lymph glands and are rather common on the lungs and heart. Any of the abdominal organs may show the characteristic hæmorrhages. The bone marrow has been reported in some cases as distinctly changed in color, the yellow marrow of long bones becoming dark red. In some cases the liver shows degeneration and necrosis or tissue death.

Typhus Fever*

Typhus fever (ship fever, jail fever) has been known to exist for centuries but until very recently we have been without precise knowledge concerning its cause. Typhus is found in all parts of the world; it affects man only and is characterized by a high fever and an eruption on the skin. The course of the disease is limited and lasts for only about twelve days. In the years 1909 and 1910 Nicolle, working in Tunis, and Anderson and Goldberger, and Ricketts and Wilder working in Mexico, showed that typhus is communicated from man to man by means of the body louse (*Pediculus vestimenti*), and that the disease is not contagious in the ordinary sense of the word. Nicolle states that after biting a typhus fever patient the louse cannot convey the infection until the fourth day thereafter and that it loses this power after the seventh day. This indicates a similarity between the microörganisms causing yellow fever, malaria and typhus. The disease may be communicated to monkeys by subcutaneous inoculations of blood

* Prepared by M. Dorset.

from a typhus fever patient. The virus may be transferred from one monkey to another indefinitely. In monkeys recovery from severe attack produces a firm immunity. No microörganism has been discovered which can be regarded as the cause of the disease. Attempts to pass the virus through filters have been unsuccessful with the possible exception of certain experiments by Nicolle. The virus is destroyed by heating from 50 to 55°.

Yellow Fever*

Yellow fever is an acute infectious, non-contagious disease of man which is seen in tropical and sub-tropical countries, particularly the West Indies, South America, and the west coast of Africa. The most notable symptoms of the disease are fever, jaundice, and hæmorrhages from the mucous membranes, this latter resulting in severe cases in what is known as "Black Vomit," which consists chiefly of extravasated blood which has been changed to a brown or black color by the action of the gastric juice.

Prior to the brilliant researches of Walter Reed and his associates on the United States Army Commission in the year 1900, it was generally believed that yellow fever was contagious, and that the disease was transmitted directly from infected to non-infected individuals, and furthermore that the clothing, bedding, and all materials which came into contact with the infected subject were capable of transmitting the disease. Reed and his associates, during the American occupation of Cuba, secured a number of volunteer subjects to serve the Commission in its studies. This Commission demonstrated positively that yellow fever could not be transmitted to man in any other way than by the bite of a particular mosquito, *Aëdes* (*Stegomyia*) *calopus* (Meigen). These mosquitoes were allowed to bite patients suffering from yellow fever at different stages of the disease. Subsequently these same mosquitoes were allowed to bite healthy men at different periods of time following their application to the infected individual. It was proved that the mosquito, in order to be capable of conveying the disease, must bite an infected individual during the first three days of the fever and at least twelve days must elapse thereafter before the mosquito is capable of transmitting the disease to a susceptible individual.

* Prepared by M. Dorset.

DISEASES CAUSED BY PROTOZOA*

Rhizopoda (von Seibold)

The amœbæ are the most important of the parasites belonging to the rhizopods. Various species of amœbæ are parasitic in the intestines of cattle, horses, mice, frogs and fish as well as human beings and most of them, like *Entamœba coli* of man, are harmless. One species, *Entamœba histolytica* (*tetragena*), produces a very severe disease of man. *Entamœba meleagridis* is the cause of a fatal disease of turkeys (page 824). *Entamœba gingivalis* (*buccalis*) is a parasite which is frequently found in a diseased condition of the gums characterized by peridental abscesses but is also frequent in apparently healthy mouths. The parasitic species lack the contractile vacuole which is a feature of the free living species that are commonly encountered so that it is not difficult to distinguish the two types.

Amœbic Dysentery

Entamœba histolytica—Schaudinn, 1903

Syn.: *Entamœba tetragena*—Viereck, 1907

Distribution.—Amœbic dysentery occurs most frequently in tropical or sub-tropical, countries, but cases of it occasionally occur in Great Britain, in Central Europe, and in the United States.

Intestinal amœbæ; Entamœba coli Lösch (Fig. 172) and *Entamœba histolytica* (*tetragena*) are both parasites in the human intestine. They measure from 15μ to 30μ in diameter and, when examined in freshly passed fæces, may be seen in active motion. Their cytoplasm contains a nucleus, vacuoles, and food particles. Both may multiply by binary and by multiple division; the appearance of certain of the encysted forms in both species indicates a process of autogamy. Both of the parasitic amœbæ of the human intestine produce a characteristic number of daughter amœbæ in the course of their multiplication. *E. coli* commonly divides into eight small amœbæ so that these organisms may present any number of nuclei below this number and occasionally they contain several more. The encysted forms of this species also divide into approximately eight small amœbæ. In *E. histolytica* the number produced as the result of division is more regular being almost invariably four whether in the division of the motile trophozoite or the encysted forms. The character of the division thus furnishes the most certain criterion in differentiating the two species. Multiplying forms are not always readily found, however, and it is necessary to take other characteristics into consideration. The non-pathogenic species (*E. coli*) is more sluggish in its movements, is generally larger, dull grayish in appearance, and with no sharp differentiation into ectoplasm and endoplasm. *E. histolytica* is active, of a greenish hue and the ectoplasm is well defined and very clear in portions extruded as pseudopods. The nucleus in the harmless species, commonly centrally situated, is larger and shows a larger amount of chromatin. The nucleus of the

* Diseases arranged generically.

* Prepared by J. L. Todd and revised by E. E. Tyzzer.

dysentery amœbæ is smaller, poorer in chromatin and commonly peripherally situated in the cytoplasm. This species also devours red corpuscles in large numbers but the absence of these in intestinal amœbæ is not sufficient basis for considering them to belong to the harmless species. The cysts are passed in the fæces; and it is through the ingestion of food or drink, contaminated by encysted amœbæ, that infection is accomplished. If unencysted amœbæ are swallowed, they are digested by the acid juices of the stomach, whereas encysted amœbæ pass through the stomach unaltered and become active in the alkaline contents of the intestine. The dysentery amœba is pathogenic to certain lower animals, and kittens have been found to be most favorable for the experimental production of the disease. Monkeys are also susceptible to a certain extent.

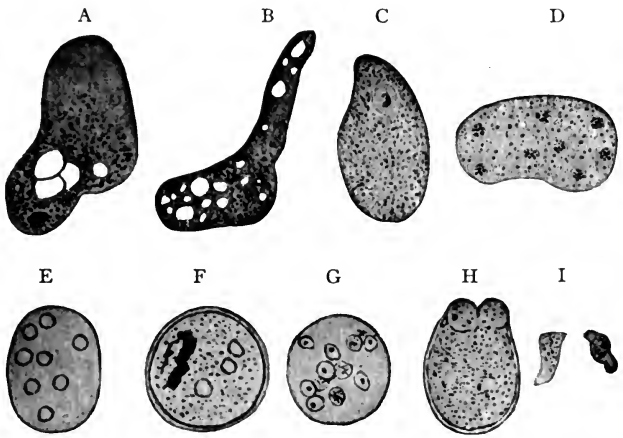


FIG. 172.—*Entamœba coli* Lösch 1875. *A-C*, various forms of motile amœbæ; *D*, the 8-nuclear stage; *E-G*, cysts with nuclear fragments; *H*, bursting cyst; *I*, young motile amœbæ. (*After Casagrandi and Barbagallo, from Doflein.*)

Entamœba histolytica may be present in an intestine for months without marked symptoms resulting. It may, however, enter the glands of Lieberkühn and pass through it into the submucosal layer of the intestine. Bacteria accompany these amœbæ and they, with the latter, cause an ulcer which spreads through a local destruction of the submucosa and undermines the mucosal layer of the intestine. In severe cases, when the ulcers have spread widely, large areas of the mucosa may be sloughed off. The amœbæ lie at the edge of the ulcer and cause it to enlarge by working their way into sound tissue; once an ulcer is started, it is not impossible that *Entamœba coli* as well as the dysentery amœbæ may be found in it. The latter live upon the red cells or fragments of intestinal cells. In chronic cases, the wall of the intestine becomes greatly thickened.

Ulcers caused by amœbæ are almost always situated in the large intestine; consequently, the symptoms of amœbic dysentery are those of inflammation of that part of the body. There is usually abdominal pain, accompanied by the passage of frequent, blood-stained stools with mucus. The infection may, however, be accompanied by no marked symptoms and there may be no diarrhœa. There are usually developed more general symptoms, such as fever and loss of flesh. The onset is frequently very gradual and insidious and the disease runs a chronic course. If amœbic dysentery causes death, it is usually does so by perforation of the bowel with resulting peritonitis, by hæmorrhage from the erosion of a blood vessel, or as the result of abscess of the liver. Liver abscesses occur not infrequently in amœbic dysentery.

Amœbic dysentery is cured with difficulty although *emetine*, a product isolated from ipecac, has recently been found of great value. Since the encysted amœbæ are killed by heat, it can be avoided by eating and drinking only foods and liquids that have been cooked.

Entero-hepatitis of Turkeys

Emtamœba meleagridis—Smith, 1895

Entero-hepatitis, or black-head, of turkeys is caused by *Entamœba meleagridis*. The disease is widespread throughout North America. It is a very fatal affection and on many farms it makes the raising of turkeys a difficult problem. The disease is characterized by thickening and ulceration of the cæca, and by extensive necrosis of the liver. *Entamœba meleagridis* is a small amœba measuring about 8μ to 10μ in diameter. Turkeys probably become infected with this parasite by swallowing its encysted forms; young turkeys may possibly become infected from encysted amœbæ, which adhere to the shells from which they were hatched.

There has been no efficient treatment devised for the disease, since it is usually not noted until far advanced, but it can be avoided through keeping healthy stock on land which has never been infected by droppings from infected birds, and by carefully wiping eggs intended for hatching with formalin.

Flagellata (Cohn emend. Bütschli)

The herpetomonads and the trypanosomes are the most important of the parasitic flagellates.

Leishmania (Ross, 1903)

The three parasites belonging to this genus which require mention are included by some authorities in the genus *Herpetomonas* but the differences with respect to habit of life justify the recognition of a distinct genus. Herpetomonads live in the alimentary tract of various insects, for example, of the common blow fly and are extracellular parasites. Their bodies in general are rigid. *Leishmania* is, on the other hand, an intracellular parasite and in the flagellated phase of its development its body is plastic and bends during locomotion. Three species are recognized in association with three distinct types of disease.

Kala Azar

Leishmania donovani—Laveran and Mesnil, 1903

This disease occurs in certain parts of Asia being first noted in Assam, Northern India.

It is caused by *L. donovani* (Fig. 173). The parasite is rarely found in the blood; when it is seen there, it almost never occurs free but is found in variable numbers

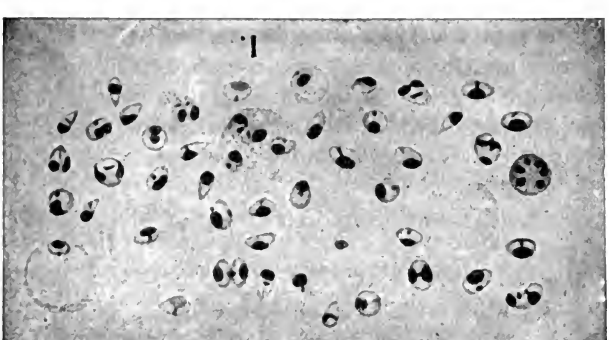


FIG. 173.—*Leishmania donovani.* Free organisms and several within cells. (*After Donovan, from Doflein.*)

within phagocytic cells. It is, usually, easily found by an examination of the juice obtained from the spleen or lymph nodes by puncture with the needle of a syringe. The liver is enlarged and it, also, contains parasites. As the organisms are seen in preparations of spleen juice, they are small ovals measuring about 2μ in length and 1.5μ in width. They consist of cytoplasm, in which lie two chromatic bodies, one of them large and rounded, the other small and rod-like. This form of the parasite may multiply in the body of the host, by binary or multiple division. If spleen pulp, or blood, containing such organisms be placed on a suitable culture

medium, they will develop in three or four days, into herpetomonad form: The large nucleus becomes the trophonucleus of the flagellate form, while th smaller, rod-like, mass becomes the kinetonucleus, from which arises the flagellun The method by which the infection is acquired is unknown; it is probably by th bite of an insect, perhaps a bedbug.

Kala azar is a chronic disease characterized by emaciation, by a irregular fever and by considerable enlargement of the spleen. Ther is great loss in strength and energy.

Although there may be periods of apparent amelioration, the diseas usually progresses steadily, in spite of treatment, to a fatal terminatior

Infantile Kala Azar

Leishmania infantum—Nicolle, 1908

Most authorities recognize the generalized leishmaniasis whic occurs in various countries bordering on the Mediterranean as a distinc disease. It is confined almost wholly to young children in whom i usually runs a fatal course. This disease has been transmitted t lower animals and the infection occurs naturally in dogs, especiall those of infested districts. Recent investigations indicate that the do furnishes a source of infection for human beings and that transmissio is affected through the agency of a species of flea.

Localized Leishmaniasis (Delhi Boil)

Leishmania tropica—Wright, 1903

The localized forms of leishmaniasis occur in widely distributed localitie throughout the tropics, and numerous local names have been applied, Aleppo Boi Oriental sore, Bagdad Boil, Biskra Button, etc. In South America likewise number of local names have been applied, Espundia, Uta, Bubas, Braziliana an Forest Yaws. In certain forms of the disease the mucous membranes are invade with loss of tissue of the nose and palate causing great deformity.

The parasites are found at the spreading edge of the lesions. As they occur i the ulcer they are oval parasites, almost identical with those which are found in th spleen of persons suffering from kala azar. If infected material be placed on culture medium, flagellated forms will be developed. In many cases the organism are difficult to find.

Delhi boil is a painless ulcer, covered by a dry scab, which usuall occurs about the face, or other uncovered portions of the body. If th

sore be left untreated, it cures itself after some months. In countries where it occurs, Delhi boil is particularly liable to form at the site of a cut or abrasion. It is possible that, in some cases, the infection may be carried to a wound by house flies.

The condition may be treated by free excision although it runs a self-limited course. In places where it is endemic, care should be taken to avoid the possibility of infection by carefully protecting all wounds, no matter how small.

Trypanosoma (Gruby, 1843)

Trypanosomes are parasitic in insects, fish, reptiles, birds, and mammals in all parts of the world. Many of them seem to be harmless parasites; others cause very serious diseases.

Sleeping sickness since it affects human beings is regarded as the most important of the diseases due to trypanosomes.

Sleeping Sickness

Trypanosoma gambiense—Dutton, 1902

Sleeping sickness is a disease of man caused by *Trypanosoma gambiense;* it is usually transmitted by the bites of *Glossina palpalis*, a tsetse fly.

Sleeping sickness occurs only in those parts of Africa where this species of fly is found.

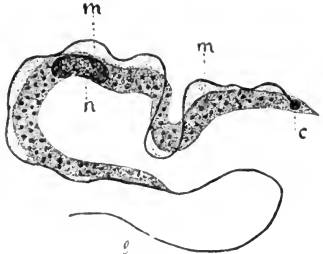


Fig. 174.—*Trypanosoma granulosum*. *n*, Tropho-nucleus; *m*, undulating membrane; *c*, kinetonucleus; *f*, flagellum. × 2000 diam. (*After Laveran and Mesnil from Doflein.*)

Trypanosoma gambiense (Fig. 174) is somewhat fusiform in shape and measures about 17μ to 25μ from the posterior extremity to the tip of its flagellum. A large

tropho-nucleus is situated near the center of the trypanosome; a smaller, kineto-nucleus lies near its posterior end. From this smaller nucleus a filament arises, which runs the whole length of the parasite and extends from its anterior end as a free flagellum. Where the filament runs along the body, the periplast extends over it to form the undulating membrane. The trypanosome moves by means of the undulating membrane and flagellum and also through the contraction of the myonemes which lie in the ectoplasm. In the blood, *Trypanosoma gambiense* multiplies by binary division. It is not impossible that it may multiply in other ways, as do other trypanosomes; for example, a trypanosome of frogs loses its locomotory apparatus and forms a sphere, then the sphere divides into many small spheres, each of which becomes a trypanosome. Sometimes *Trypanosoma gambiense* loses its locomotory apparatus and forms a sphere; these forms are found in the organs of infected animals. They are probably more resistant, resting forms and a single trypanosome may be formed from some of them.

Trypanosomiasis is easily transmitted to susceptible animals by inoculation. It is possible that the disease may be transmitted occasionally, in this way, by the mere

FIG. 175.—*Trypanosoma gambiense.* (*After Minchin, from Doflein.*)

mechanical exchange of infected material, through an insect's bite, from an infected to a healthy individual. But, as a rule, the disease can only be transmitted by the bites of *Glossina palpalis* in which the organism has developed for some time (Fig. 175); this fly is not usually infective until three weeks after it has fed on an infected person, and it retains its infecting power for some months.

An incubation period of at least ten days intervenes between the bite and the appearance of symptoms, but this period may be much longer, for trypanosomiasis may manifest itself in apparently healthy negroes several years after they have left localities in which the disease could have been acquired. The disease sometimes causes death within three or four months; but it may last for one or more years. It is a chronic, wasting affection, characterized by loss of strength and energy, and by an irregular fever. A change in the

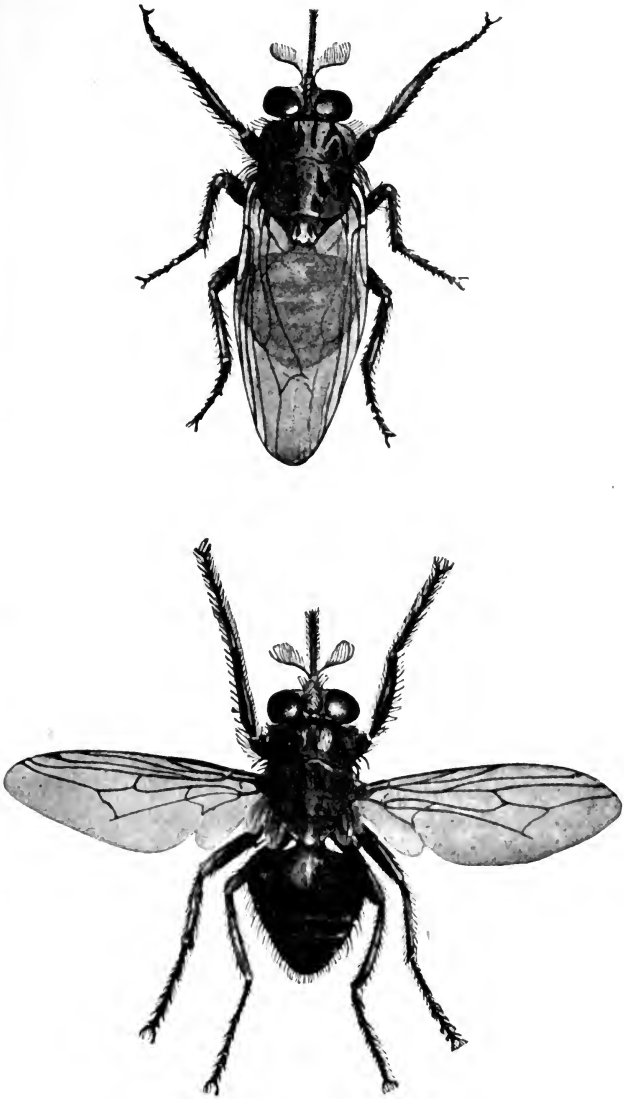


FIG. 176.—*Glossina palpalis.* (*After Doflein.*)

mentality, red blotches on the skin, and enlargement of the lymphatic glands, are all early signs of the disease. In the later stages, headache, mania, uncontrollable sleep, and other nervous symptoms may be present. Death rarely results from trypanosomiasis alone; the patients usually succumb to one of the secondary infections, to which their reduced condition makes them especially liable. No toxin has been isolated in trypanosomiasis, but from the nature of the infection no great amount would be necessary to produce symptoms. The parasites not only live in the blood and other fluids of the body but are found in the tissues of various organs. They are distributed throughout the tissue of the brain and their presence is associated with infiltration of the tissue about the blood-vessels with large numbers of lymphocytes.

The recognition of trypanosomiasis depends upon the demonstration of the parasites. They may be found in fresh or stained preparations of the blood, in the juice obtained by aspirating an enlarged lymphatic gland, or in the cerebrospinal fluid. The examination of the blood is the simplest method of searching for trypanosomes; the examination of gland juice is the most efficient one.

The improvement in the methods of treating trypanosomiasis during the past ten years (1901-1911) affords an excellent example of the value of laboratory work. Before 1901 arsenic, given in some inorganic form, was the only drug known to have any effect on trypanosomiasis. Inorganic arsenic drives the parasites from the blood and improves the patient's condition. Unfortunately, the trypanosomes usually reappear and, then, they have become resistant to arsenic so that the patient succumbs in spite of repeated doses of the drug. Many organic compounds of arsenic were experimented with in the hope of finding an efficient trypanocide and several valuable drugs have been found: "Atoxyl" which is the sodium salt of para-amido-phenyl-arsenic acid, acetylated atoxyl, and arsenophenylglycin, are all organic compounds of arsenic. They are much more effective than is arsenic itself. Similar organic compounds of antimony and tartar emetic are almost as effective, while certain aniline dyes have a distinct trypanocidal value. It has been found that trypanosomes may become resistant to any one of these drugs, and that drugs may destroy some stages of the trypanosome while they are unable to destroy others. In order to give the parasites no opportunity of acquiring resistance to any drug, and in

order to destroy them at all stages of their development, the following general rules are now observed in the treatment of trypanosomiasis. The drugs employed should be alternated, and they should be given as early in the disease as possible, and in as large doses as possible. It is probable that these principles will be found to be of value in the treatment of other diseases caused by protozoa.

The prevention of the disease depends upon the avoidance of the water's edge, where *Glossina palpalis* exists, and of the proximity of persons infected by trypanosomiasis. The most usually successful way of recognizing infected persons is by the discovery of trypanosomes in the fluid aspirated from their enlarged lymphatic glands.

Human Trypanosomiasis of South America

Trypanosoma cruzi—Chagas, 1909

This disease is caused by *Trpyanosoma cruzi* (*Endotrypanum cruzi*) and is transmitted by the bites of a reduviid insect, *Conorrhinus megistus*. It has only been found in Brazil.

Trypanosoma cruzi may be either free in the blood plasma or lie within a red cell. It multiplies, in the organs, by losing its locomotory apparatus and forming a sphere which divides into eight portions: a new trypanosome develops from each portion.

The disease is a chronic one, characterized by irregular temperature, by wasting, œdema, and enlargement of the spleen and lymphatic glands. It occurs chiefly in young children and is often fatal. It may be prevented by avoiding the insect which transmits it—the habits of the *Conorrhinus* resemble those of a bed bug.

Trypanosomiases of Animals

Several diseases, of great economic importance, which affect domestic animals, are caused by trypanosomes. The following are the most important. Tsetse-fly disease, or nagana, of Southern Africa, is caused by *Trypanosoma brucei* (Plimmer and Bradford) and it is transmitted by the Tsetse fly, *Glossina morsitans;* it affects all domestic animals.

In South America, Mal de caderas, a disease of horses, is caused by

Trypanosoma equinum (Voges); it is probably transmitted by a biting fly, *Stomoxys*.

All through Asia, Surra, caused by *Trypanosoma evansi* (Steel), is a severe disease of cattle and equines; it is probably transmitted by a biting fly.

Trypanosoma dimorphon (Laveran and Mesnil) and many other trypanosomes, more or less closely allied to it, cause diseases of horses, cattle, and other domestic animals in many parts of Africa; they are probably all transmitted by the bites of flies.

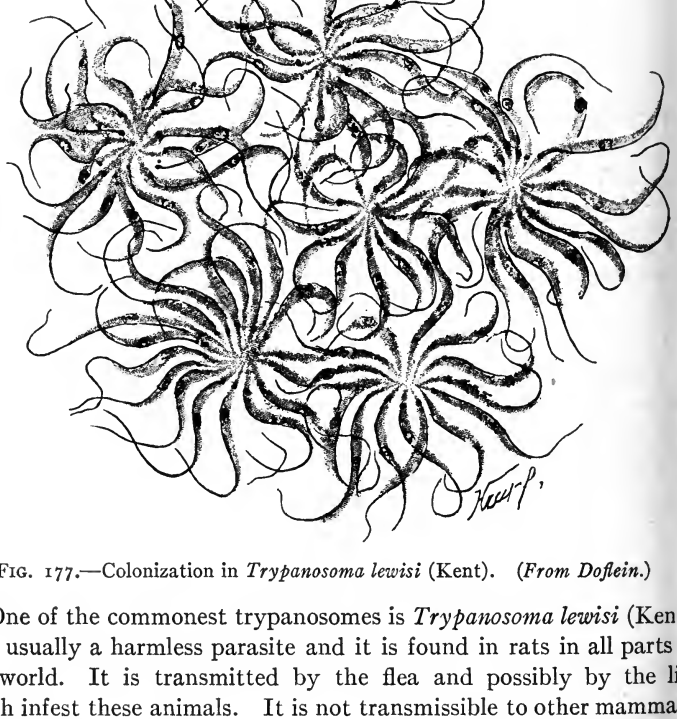


FIG. 177.—Colonization in *Trypanosoma lewisi* (Kent). (*From Doflein.*)

One of the commonest trypanosomes is *Trypanosoma lewisi* (Kent). It is usually a harmless parasite and it is found in rats in all parts of the world. It is transmitted by the flea and possibly by the lice which infest these animals. It is not transmissible to other mammals.

Dourine or maladie de coit, is a serious disease of equines; it is caused by *Trypanosoma equiperdum* (Doflein). This disease was

brought to North America by an imported Percheron stallion. It is now endemic in some of the western states and in part of southern Alberta, in Canada. It is transmitted by coitus and, perhaps, rarely by the bites of fleas.

A very large trypanosome, *Trypanosoma theileri* (Bruce), occurs in cattle in southern Europe and in Africa and a large trypanosome, *Trypanosoma americanum*, resembling this one, has been found in cattle in the United States. These trypanosomes seem to do no harm to their hosts.

Although there are slight differences, the symptoms are much the same in all the trypanosomiases of animals, and they much resemble those which occur in the diseases produced in men by trypanosomes. Occasionally, as in nagana, an animal trypanosomiasis may run an acute course, and kill the host in two or three weeks, but usually, they are diseases of long duration, characterized by irregular fever, œdemas and progressive loss of strength, weight, and energy. Localized areas of œdema beneath the skin and about the genitals are especially seen in dourine; *Trypanosoma equiperdum* is most easily found by examining serum obtained by puncturing these œdemas.

Sporozoa (Leuckart, 1879)

This class contains many very important pathogenic parasites.

Coccidia (Leuckart)

Coccidia of various species are parasitic in the epithelial cells lining the intestines of mice, horses, cattle, pigs, goats, and other animals. In Europe, *Eimeria stiedæ* (*Coccidium cuniculi*) sometimes causes an enteritis of cattle; in East Africa, a coccidium causes a serious disease of cattle. Other coccidia kill many young pigeons, grouse, and chickens. Coccidia have been reported as infecting the intestinal tract of man.

Coccidiosis of Rabbits

Eimeria stiedæ—Lindemann, 1865

Syn.: *Coccidium cuniculi*

The coccidium causing this disease is the best known of the coccidia infecting mammals.

This coccidium is parasitic within the epithelial cells of the intestine and within the epithelium lining the bile ducts. Adult, asexual forms measure from 20μ to 50μ in diameter and they produce from 30 to 200 merozoites. The merozoites infect other epithelial cells where they may again multiply asexually or they may develop into male and female forms destined to multiply sexually. One of the micro gametes, produced by a microgametocyte, fertilizes a macrogamete and an oöcyst is developed. Within the oöcyst a number of sporoblasts form, which contain two spores each. The oocysts are passed with the fæces and if they are ingested by a suitable host the spores are set free. When the cyst reaches the intestine, the sporozoits are liberated, and a new infection is commenced.

Since the cells parasitized by the coccidia are destroyed, it is evident that a severe infection may do a great deal of harm and interfere with the functions of both intestine and liver. The disease may be limited by making it impossible for uninfected animals to come into contact with the droppings of infected stock.

Avian Coccidiosis

Coccidium infection is of frequent occurrence among birds and especially those of domestic varieties without causing serious symptoms. It is known, however, to cause severe epidemics in certain species, and when present in milder form should be regarded as antagonistic to health. *Entamœba meleagridis* the organism of "Black head" in turkeys from its peculiar relationship to the tissues has been erroneously regarded as a form of coccidium.

Hæmosporidia (Danilewsky emend. Schaudinn)

The most important parasites of this order are those, belonging to the Genus *Plasmodium*, which cause malaria in man. Organisms similar to these are parasitic in the red blood cells of higher apes and monkeys. *Proteosoma* and *Hæmoproteus* are two genera parasitic in the red blood cells of birds. It was the study of these avian parasites which led to the discovery of the way in which malaria is transmitted by the bite of the mosquito.

Plasmodium (Marchiafava and Celli)

At least three species of this genus are parasitic in man: *Plasmodium vivax* (Grassi and Feletti), the cause of tertian malaria, *Plasmodium malariæ* (Laveran), causing quartan malaria, and *Plasmodium falciparum* (Welsh), which causes aestivo-autumnal malarial fever.

Malaria

Malaria is a disease caused by an amœboid parasite of the red blood corpuscles and is transmitted by the bite of anopheline mosquitoes in which the parasite has completed the sexual cycle of its development. It exists in all parts of the tropical and subtropical world (Fig. 178).

A young malarial parasite or sporozoit, derived from the mosquito enters a red cell and supports itself by living upon the cell's substance. The parasite grows, proceeds to multiply asexually and divides into a number of merozoites which are set free

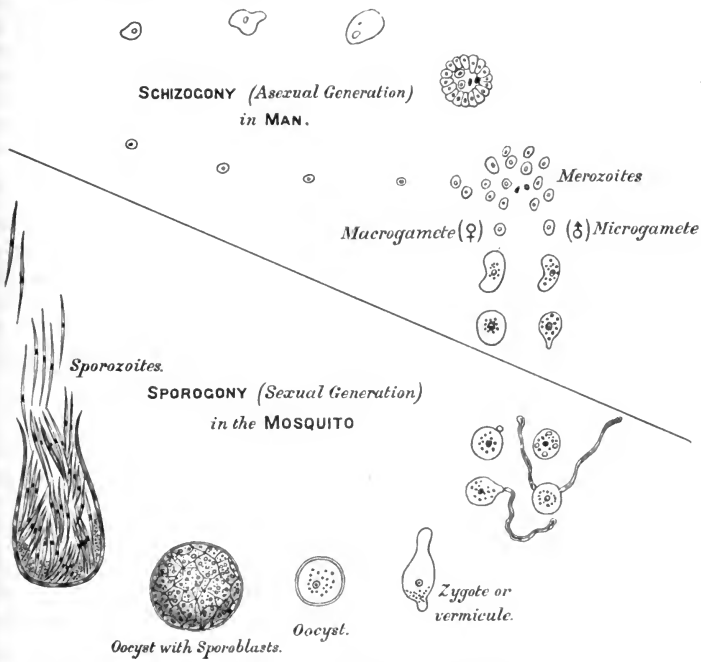


FIG. 178.—Diagram illustrating the human and mosquito cycles of existence of the malaria parasite. (*After Martin's General Pathology.*)

by the rupture of the red cell. Those of the merozoites which escape ingestion by the white cells of the blood enter red cells where they may again multiply asexually, or they may develop into sexual forms. When blood, containing malarial parasites, is ingested by a suitable mosquito, all the parasites, except the adult sexual ones, are digested and die. Soon after they are ingested, the macrogametocyte extrudes polar

bodies and become a macrogamete and the microgametocyte produces several microgametes, one of which enters and fertilizes the macrogamete. Through the fusion of macrogamete and microgamete a copula is formed, which since it is motile is called an oökinet. This makes its way until it comes to lie just beneath the outer surface of the mosquito's stomach. There it develops, as an oöcyst, until it reaches several times its original size. It divides into a number of areas, or sporoblasts, each of which subdivides to form many very small, hair-like sporozoites. When the oöcyst bursts, some of the sporozoites pass forward to find their way into the salivary glands of the mosquito, and, when it bites, they are extruded, with the saliva, into the body of the person from whom blood is being sucked. The entry of a sporozoite into a red cell recommences the cycle of development which has just been described. If the adult sexual parasites are not taken up by a mosquito they die off in the blood, but some of the female forms may live for years and then divide parthenogenetically, without a precedent fertilization, to produce several young parasites. It has been suggested but never demonstrated that the sporozoites may enter eggs lying in the ovaries of infected mosquitoes and that mosquitoes, hatched from such eggs, may inherit the infection from their parent and that they, also, are able to transmit malaria.

In fresh preparations of blood, a malarial parasite is seen as a body of varying size, which is more refractile and of a lighter color, than the red cell which contains it. In its growing phase it has distinct amœboid movement and the pigment granules lying in it are in active motion. In preparations, stained by a modification of Romanowsky's method, every malarial parasite is seen to possess a definite purple nucleus surrounded by blue-staining cytoplasm. Young parasites measure less than a fifth of the diameter of a red cell in width; adult parasites may completely fill the cell which contains them. Malarial pigment is the waste product which results from the digestion of the hæmoglobin of the red cells by a malarial parasite, and consequently, since they have digested more hæmoglobin, the older parasites contain more pigment than do the younger ones. A mature asexual finally segments into a number of merozoites; *Plasmodium vivax* forms about eighteen, *Plasmodium malariæ* about eight merozoites. The adult sexual forms of *Plasmodium falciparum* are shaped like a crescent. The three malarial parasites of man may be distinguished from one another by these peculiarities as well as by other, lesser differences in themselves and in the red cells which they parasitize.

When a mature, asexual, malarial parasite bursts, it sets free young parasites and a toxin. Practically all of the parasites, present in a person suffering from typical acute malaria, mature and burst at the same time and the considerable amount of toxin, set free in this way, produces a paroxysm characterized by chills and fever. The parasites of *Plasmodium vivax* mature in forty-eight hours. Consequently, a person infected by it has a chill when schizogony occurs, on every third day, and the disease caused by it is called a *tertian fever*. *Plasmodium malariæ* matures in seventy-two hours, causes

DESCRIPTION OF PLATE I.

(Reproduced from Greene's Medical Diagnosis)

Plasmodia of three varieties. Stained by Wright's stain.

In this plate the chromatin of the parasites is shown in red while the pigment granules appear as black dots.

THE QUARTAN PARASITE (*P. malariæ*)

1–9. Asexual multiplication. 10. Adult gametocyte. 11. Normal red cell. 12. Flagellating microgametocyte.

THE TERTIAN PARASITE (*P. vivax*)

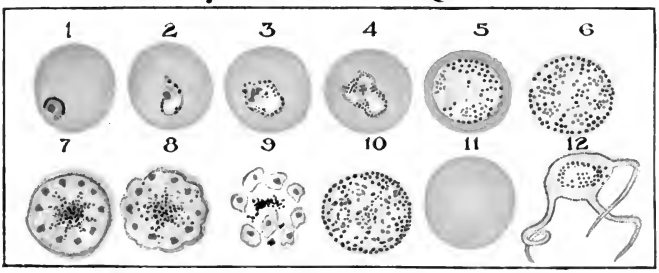
13–21. Asexual multiplication. 22. Flagellating microgametocyte.

THE ÆSTIVO-AUTUMNAL PARASITE (*P. falciparum*)

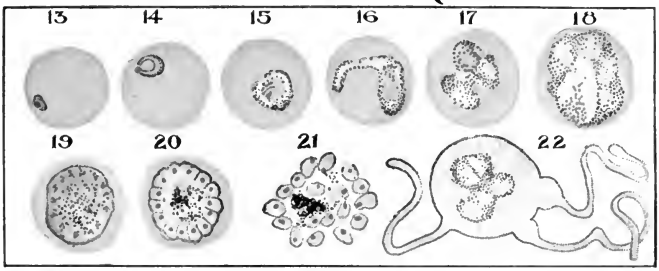
23–31. Asexual multiplication; 25 and 26 are doubly infected cells.
32–35. Gametocytes. 36, 37. Flagellating microgametocyte.

PLATE I.

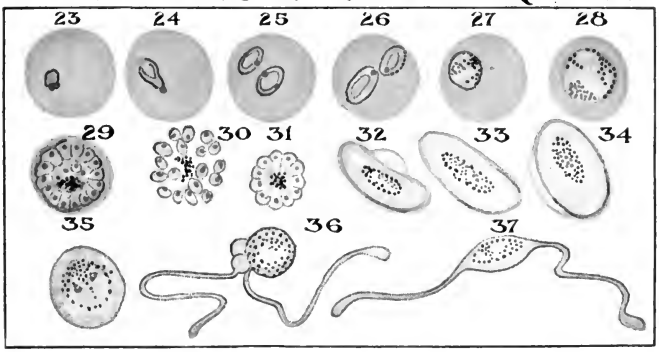
THE QUARTAN PARASITE



THE TERTIAN PARASITE



THE ÆSTIVO-AUTUMNAL PARASITE



an attack of ague on every fourth day and the disease produced is called *quartan fever*. Patients infected by *Plasmodium falciparum* often have a *quotidian fever* with a daily rise in the temperature, although a three day period may be recognized in some cases. The disease is called æstivo-autumnal fever. There are three stages in the paroxysm: during the chill, the patient feels cold; in the hot stage he feels warm—his temperature is above normal during both stages; in the sweating stage the temperature falls to normal and the patient's discomfort becomes much less.

The regularly recurring chills and fever constitute the only symptoms characteristic of malaria and a regular rise in temperature on the third or fourth days of an illness is strongly suggestive of a malarial infection. The type of disease and the symptoms, produced by a malarial infection, may vary almost indefinitely according to the precise way in which the host is harmed by the infection. Consequently, an enumeration of the clinical manifestations of malaria is of less importance to a student than is an understanding of the way in which the malarial parasites harm their host. The malarial parasites destroy the red cells and thus cause an anæmia with the symptoms which result from it. Secondly, they produce toxins which may cause both acute and chronic intoxications. The acute intoxications are seen in the elevation of body temperature and in unconsciousness in some pernicious forms of malaria; malarial neuritis is an example of chronic intoxication. Lastly, malarial parasites may do harm by blocking the capillaries and causing the death of the cells which are cut off from the circulation, the symptoms which result depending upon the functions of the cells which are destroyed. If the disease be long continued, with a high temperature, the degenerative changes which usually result from chronic disease and constant fever are produced in the patient.

The definite diagnosis of malarial fever depends upon the demonstration, in a patient, of the malarial parasite, or of the pigment produced by it.

Quinine has a specific action on the malarial parasite and is the most valuable drug available for the treatment of the disease. It must be given in full doses and treatment continued until all parasites disappear from the blood.

Malaria, since it is of the type of disease which is produced by

an insect-transmitted parasite, may be prevented by measures directed either against the parasite or against the transmitting agent. Malaria is caused by a *Plasmodium* and transmitted by the bites of mosquitoes belonging to the *Anophelinæ*. The disease may be combated by destroying the parasite, in infected persons with quinine, and by isolating such persons under mosquito nets so that mosquitoes may never have an opportunity of ingesting the parasites which they harbor in their blood. Malaria may also be prevented by destroying the mosquitoes which transmit it. The most efficient way of getting rid

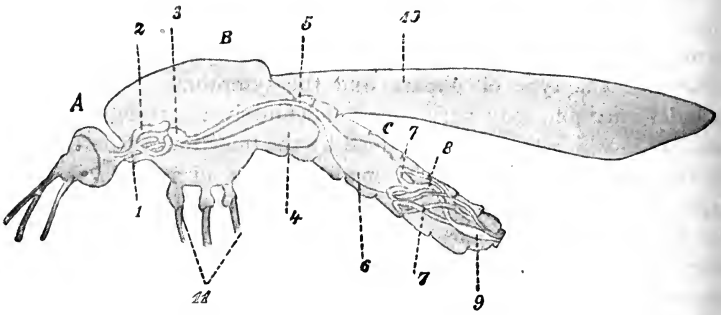


FIG. 179.—Longitudinal section of *Anopheles*. *A*, head; *B*, thorax; *C*, abdomen 1, œsophagus; 2, salivary glands; 3, dorsal reservoir; 4, ventral reservoir; 5, cana entering stomach; 6, stomach; 7, malpighian tubes; 8, hind-gut; 9, rectum; 10, wings 11, legs. (*After Grassi, from Lang and Doflein.*)

of mosquitoes is to make it impossible for them to breed. The eggs of a mosquito are laid in water, and water is absolutely necessary for the larval and pupal stages, which must be passed through before the adult mosquito is produced. Fish destroy developing mosquitoes and large sheets of water are also too rough for them—so mosquitoes must have, for breeding, rather small collections of fresh water free from fish. Mosquitoes will soon disappear from a locality if all such collections of water, within a quarter of a mile of it, are filled up, drained, or covered with a film of coal oil so as to make it impossible for the mosquitoes to breed in them. Those who live in a malarious district should protect themselves from mosquito bites by the careful use of mosquito-netting. By the simple observance of these evident indications, malaria has already been banished from several localities in which it was formerly endemic.

Babesia (Starcovici, 1893)

This order is often called PIROPLASMA. It includes several species of parasites, which cause diseases of considerable economic importance in horses, cattle, sheep, and dogs. One of the best-known species is *Babesia bigemina*, which causes Red-Water or Texas Fever of cattle. The parasites which are associated with the numerous babesiases are distinguished from one another by the host in which they are found, by slight differences in their morphology and by their inoculability into various animals.

Red Water

Babesia bigemina—Smith and Kilborne, 1893

Red water is one of the names given to a disease of cattle which is characterized by hæmoglobinuria; in the United States it is often called Texas cattle fever. It is caused by *Babesia bovis* (*bigemina*) (Fig. 180). The parasite is transmitted by the bites of a tick, in North America, by *Rhipicephalus annulatus*.

Red water occurs not only in the southern portion of the United States but almost everywhere in the tropics and in many of the warmer parts of the temperate zones.

The parasite is a pear-shaped organism which usually lies within a red cell. It measures from 2μ to 4μ in length and about 1μ in breadth. In fresh preparations they appear as refractile bodies possessed of slight amœboid movement; in stained preparations they are seen to consist of a blue-staining cytoplasm which contains a mass of chromatin at its broader end. Multiplication is accomplished by simple division into two or more parts; it is possible that schizogony and sporogony may also occur. The parasites are often very scarce in the peripheral circulation but are much more numerous in the organs and particularly in the spleen. The disease can be transmitted, experimentally, from bovine to bovine by the inoculation of blood which contains parasites; normally, it is transferred from animal to animal by the bites of a tick. The species of tick which carries red water is not the same in all parts of the world.

Ten days intervene between the bite of the infecting tick and the first sign of the infection. The temperature rises, it may be, to 106°, or more, and it remains high for a week. The animal is evidently very ill, it has no appetite, and it rapidly loses strength and weight. Many red cells are destroyed and anæmia may be marked. The urine is albuminous and it is red because of the hæmoglobin which it contains. Death may occur in very acute cases as early as the second day. Animals which recover from a severe attack are usually immune to the

disease. The immunity is not an absolute one, however, for blood taken from such recovered animals is often infective; the parasite probably exists in them in a latent form through the establishment of a tolerance on the part of the host.

There is no specific treatment for babesiosis. Some of the aniline drugs, used in the treatment of trypanosomiasis, such as trypan-blue, are of some value.

Many districts are kept free from red water by not allowing cattle coming from infected districts to enter them. Where it exists, the disease is controlled by destroying the ticks on cattle with poisonous

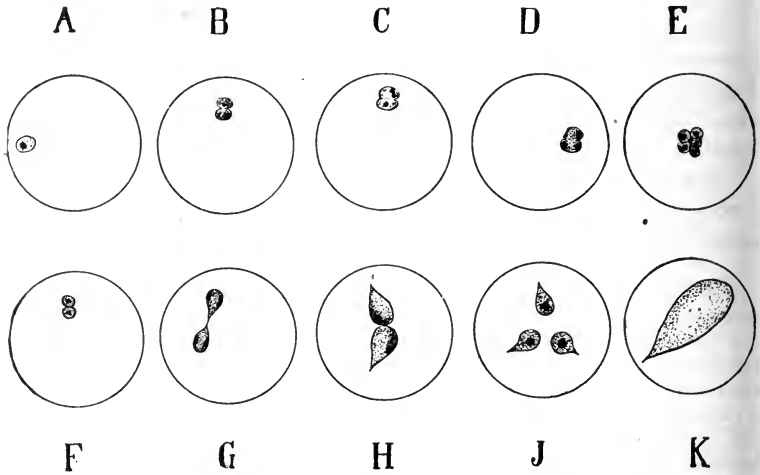


FIG. 180.—*Babesia bigemina*. Various stages of development in red blood cells. *A*, young parasite; *B*, a twin-form; *C-E*, a multiple division; *F-K*, large pear-shaped forms. (*After Doflein.*)

washes and by occasionally plowing, or burning over, the pastures in order to destroy ticks which have dropped to the ground. In the United States, cattle on some farms are kept free from ticks, and consequently from red water, by a manœuvre which takes advantage of the way in which the tick transmits the disease. The adult tick remains upon her host until she is ready to deposit her eggs; she then drops off, lays her eggs and dies. The young ticks, hatched from these eggs, attach themselves to new hosts and it is through their bites that the disease is transmitted. Therefore, since the disease is transmitted by

the progeny of ticks which have fed upon infected mammals, susceptible cattle may be protected from the disease by preventing young ticks from reaching them. This may be done by not allowing them to feed over fields where ticks may have been dropped until sufficient time, about ten months, has elapsed for all the ticks and their progeny to have died of starvation.

There are a number of parasites which although closely related to the Babesias are usually placed in other genera. Most of these have been shown to be transmitted by ticks of various species.

East Coast Fever

Lympho-hæmocytozoön parvum—Meyer (Theiler, 1903)
Syn.: *Theilerio parvum*

The parasite causing this disease which is characterized by severe anæmia is found in the red blood corpuscles of infected cattle. The intracorpuscular forms vary in form, some being slender and rod-shaped, the others being more rounded or pear-shaped. They may be arranged to form a cross, but this is not due to segmentation but to fortuitous grouping in heavily infected cells. They are regarded as gametocytes, for although such blood is infectious for ticks it will not produce infection when injected into normal cattle. The multiplicative or asexual phase of the organism is restricted to certain organs, especially the lymph nodes, spleen and bone marrow. The tissue from these organs when injected into normal cattle produces infection.

Theileria (*Babesia*) *mutans* is a parasite of cattle closely resembling *T. parvum* since it furnishes many rod-shaped forms in the red blood corpuscles. The cross-forms which occur in this species result from segmentation and since the blood is infectious when injected into normal animals it is evident that the asexual phase is present in the blood. This parasite produces no serious symptoms and does not appear to affect the health of the animal.

Oroya Fever

Bartonella bacilliformis—Strong, Tyzzer and Sellards, 1915

A human disease characterized by rapidly developing and severe anæmia associated with an irregular fever occurs in certain mountain valleys in Peru. The red blood corpuscles harbor slender rod-shaped

and small rounded organisms in numbers varying with the severity of the disease. The rods are frequently arranged in chains of two, three or even four, and present deeply stained granules at one or both extremities. Examined in fresh preparations they are found to move slowly without marked change of shape through the interior of the red cell. Cross-forms are rare and probably represent fortuitous arrangement rather than segmentation of the organisms. The endothelium of the blood-vessels of the lymph nodes, spleen and liver contain organisms in various stages of development, small rod-shaped forms similar to those of the red cells eventually being formed. The distention of the endothelial cells is often sufficient to occlude many of the blood-vessels and in the lymph follicles of the large intestine this has apparently led to the necrosis of the surrounding tissue and ulceration. The organism of this disease is smaller than that of East Coast fever, the rod forms being very slender and nuclear material is not so readily differentiated. Its resemblance in other respects together with the similarity of its distribution in the tissues indicates a relationship to this group of organisms.

The disease has not been transmitted to lower animals. Carrion, a Peruvian student, who inoculated himself with the blood of a patient suffering from Verruga peruviana died from a disease which may have been Oroya fever, although the evidence on this point is inconclusive. Oroya fever and Verruga peruviana not infrequently occur simultaneously in the same individual, just as the latter disease is frequently complicated by malaria, and this together with the result of Carrion's experiment led many Peruvian physicians to the erroneous belief that Oroya fever and Verruga were different stages or manifestations of a single infection. Verruga is, however, readily transmitted to lower animals. The mode of transmission of Oroya fever has not been conclusively determined.

Anaplasmosis

In a pernicious anæmia of cattle and at times in babesiasis the red blood corpuscles contain minute deeply stained, rounded bodies. These are frequently in pairs and are commonly situated near the margin of the cell so that they have been given the name *Anaplasma marginale* (Theiler), by those who are convinced of their parasitic nature. They have also been found in other domestic animals and similar structures

are found normally in all individuals of certain species, as for example, the mouse. Since the bodies of this general appearance which occur in normal animals are evidently to be regarded as nuclear material certain investigators are inclined to doubt the parasitic nature of *Anaplasma*.

Sarcosporidia (Balbiani)

Different species of this order are frequent parasites of all the domestic animals, of mice and, occasionally, of man. Mice are killed by them and it is possible that they may produce ill effects in men and domestic animals but no definite disease is associated with their presence. Though they may occur in any part of the body, they are most numerous in certain muscles, such as those of the larynx and œsophagus, which are near the alimentary canal. For this reason it seems possible that they may enter the bodies of their hosts with food, but our knowledge of their life history is incomplete.

Myxosporidia (Bütschli)

There are many species in this group which are parasitic in fishes and certain arthropods but not in higher animals. The classification is based largely on the character of the spores produced. The latter are provided with a resistant membrane or shell and with polar capsules, each of which contains a coiled filament which when extruded serves to anchor the spore to the surface. The spores are produced continuously within the protoplasm of the mother organism which may be situated in any part of the body of the host. Severe infection with myxosporidia may cause boil-like lesions and the death of large numbers of fishes.

Microsporidia (Balbiani)

Protozoa belonging to this order do not produce disease in man. They are the cause of a disease of bees, and they are of particular interest because one of them, *Nosema bombycis*, causes Pébrine, a serious disease affecting silk-worms (page 656).

Infusoria (Leddermüller, 1763)

Most of the parasitic infusoria occur in the alimentary tracts of their hosts. Harmless infusoria are found in the stomachs of many

herbivorous animals and also in the large intestine of the frog. *Balantidium coli* is a common and apparently innocuous parasite of the cæcum and large intestine of the pig, but it may cause a severe inflammation of the large intestine in man which not infrequently proves fatal. One or two other infusoria occasionally produce similar symptoms in man. Other species of infusoria are parasitic on fish. Some of these are harmless, but some by finding their way into the gills or beneath the scales, cause serious diseases.

Balantidium Enteritis

Balantidium coli—Malmsten, 1857

Balantidium coli is the most important of the infusoria parasitic in man and may cause a form of dysentery.

This organism measures about 150μ in length and 50μ in breadth. It is covered with cilia; its cytoplasm is differentiated to form oral and anal areas and it contains digestive and contractile vacuoles. It multiplies by simple transverse division either with or without a precedent conjugation. It may encyst, and this is the form in which the parasite is transmitted from one host to another.

High enemata of mild antiseptics have been used in the treatment of this infection.

Parasites of Uncertain Position

In Panama, there is a disease of man, somewhat resembling one form of tuberculosis, which is caused by a protozoön called *Histoplasma capsulatum*. The only known stage of this parasite greatly resembles the non-motile form of *Leishmania donovani;* but it contains only one not two masses of chromatin. This organism is certainly a protozoön although the genus to which it belongs has not been determined.

Chlamydozoa (Prowazek, 1907)

This name is given to certain bodies because their presence excites the cell containing them to produce a substance which surrounds them like a cloak. The exact nature of these bodies is disputed; it is even doubtful whether they are parasites, or whether they are merely the expression of some morbid change, produced in the cells, by an unseen virus which causes the disease. They have been found in trachoma, a disease of the eyelids of man, in hydrophobia, in *Molluscum contagiosum*, a skin disease, in smallpox, in vaccinia, and in scarlet fever. They are mentioned with the protozoa because, if they are parasites, they are probably more nearly allied to the protozoa than to the bacteria. They are extremely small bodies, some measuring only 0.25μ in diameter. They are spherical and occur within

the cells. In preparations stained by Romanowsky's method they are colored like chromatin.

ULTRAMICROSCOPIC VIRUSES (See page 116)

SPIROCHÆTA (Ehrenberg, 1833)

Many spirochætes are, apparently, harmless parasites in shell fish, in the alimentary canals of various animals and in the blood of fish, birds, and many mammals; other spirochætes produce disease in men and in lower animals.

Several spirochætes are parasitic in man. *Spirochæta dentium* and *Spirochæta buccalis* are harmless organisms which are found in tartar, about the teeth.

Spirochæta vincenti occurs in great numbers in a certain form of sore throat. Other spirochætes have been found in foul ulcers, and others have been found in association with bronchitis and enteritis. All these are comparatively unimportant parasites. *Spirochæta duttoni, Spirochæta obermeieri and Spirochæta pallidula* are more important ones.

AFRICAN TICK FEVER

Spirochæta duttoni—Novy and Knapp, 1906

African tick fever is a disease caused by *Spirochæta duttoni* and transmitted by the bites of a tick *Ornithodoros moubata* (Fig. 181).

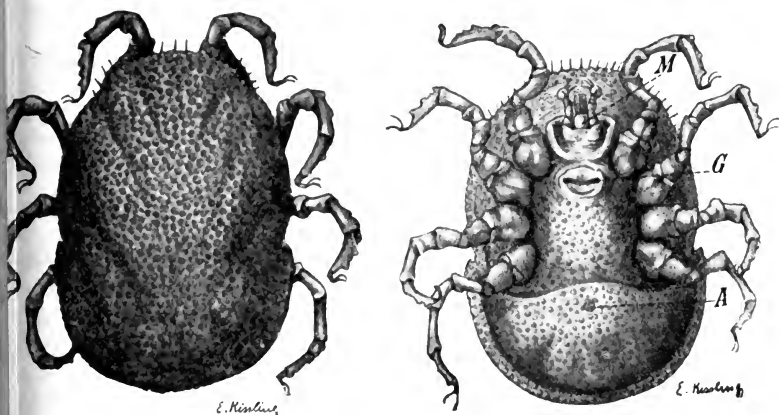


FIG. 181.—*Ornithodoros moubata.* (*Murray from Doflein.*)

This disease exists in Central and Eastern Africa, wherever *Ornithodoros moubata* occurs. A disease, which is probably caused by a spirochæte, is transmitted by another tick, *Argas persicus*, in Persia.

In Central America a spirochæte, which causes a disease almost identical with tick fever, is carried by *Ornithodoros chinche.*

Spirochæta duttoni is a slender organism measuring from 14μ to 16μ in length; its thread-like body lies in a number of waves, which vary greatly in number, according to the way in which the preparation is made; consequently, the number of waves is not a constant character which can be relied upon for the identification of this species of spirochæte. This spirochæte is composed of an outer ectoplasmic sheath, and of an interior composed largely of chromatin; the sheath extends at either end into flagellum-like prolongations. Multiplication is accomplished by transverse binary division. Sometimes, perhaps most often toward the end of an attack of fever the spirochætes coil up tightly, within a cyst-like matrix. Such encysted forms may lie within cells, *i.e.*, liver cells, and spleen cells; they are seen most frequently in the liver and spleen, and they are always present in the alimentary canal of ticks which have ingested spirochæte-infected blood. The chromatin of both free and encysted spirochætes may be fragmented, more or less regularly. In the tick, cysts, containing a spirochæte with fragmented chromatin, burst and set free the granules of chromatin. Some investigators believe that each granule develops into a spirochæte, others that this represents a degeneration and destruction of the parasite. It is not impossible that some such method of multiplication occurs in man.

FIG. 182.—*Spirochæta duttoni.* (*After Doflein.*)

The form and the exact way in which the spirochæte is transmitted by the tick is not known. It is probable that a tick, once infected, never loses its power to transmit the disease; the infection may be transmitted, from mother to daughter, through at least three generations of ticks.

The ticks hide during the day and feed at night. The wound produced by their bite is insignificant.

An incubation period of about five days intervenes between the tick bite and the appearance of symptoms. The fever is characteristic; it rises rapidly to, perhaps, 105° and it remains high for from three to five days. It then falls suddenly and there is no fever for from five days to two weeks. Then the temperature rises again and there may be from three to six such recurrences of fever before the illness ends. The definite periodicity of the relapses probably depends upon some more or less regular developmental change in the spirochætes since the latter are always most numerous in the blood during the height of the fever. The disease is not often fatal and "606" is a specific treatment for it. It can be easily prevented by avoiding tick bites.

Relapsing or Recurrent Fever

Spirochæta recurrentis—Lebert, 1874

This is still a common disease in some parts of Europe. Its symptoms are almost identical with those of tick fever; and the spirochæte causing it, formerly called *Spirochæta obermeieri*, can only be distinguished from *Spirochæta duttoni* by the fact that an animal which has recovered from an infection by one of these parasites is immune to inoculation with it, but is susceptible to an inoculation with the other spirochæte. The means by which relapsing fever is transmitted is not known; it is probably carried by the bites of lice, or bedbugs.

Treponema (Schaudinn, 1905)

Two species of this genus are very important parasites.

Syphilis

Treponema pallidum—Schaudinn, 1905

This disease, in all its diverse forms, is caused by *Treponema pallidum.*

The treponema is an exceedingly slender, thread-like organism, with a waved body which measures from 6μ to 14μ in length (Fig. 183). It greatly resembles the spirochætes, but differs from them in having each end drawn out to resemble a very slender flagellum. Very little is known of the life history of the treponema except that it multiplies by transverse division. It is transmitted by the contact of a lesion, containing the parasites, with the broken skin, or with a mucous membrane of an uninfected person. The symptoms of syphilis are attributable to continuous mild injury, and destruction of the tissues of the infected persons by the treponema and by its toxic effects. It is a parasite of the connective tissue.

Mercury and potassium iodide were formerly almost exclusively employed in treating syphilis. The search for an efficient drug for the treatment of trypanosomiasis has led to the discovery of other drugs which are of value in the treatment of syphilis, such as atoxyl (the sodium salt of para-amido-phenyl-arsenic acid) and its acetylated derivative, and of dichlorhydrate-diamido-arseno-benzol. The last-named drug has proved of great importance in the treatment of syphilis.

Yaws or Framboœsia

Treponema pertenue—Castellani, 1905

This is a disease of the tropics which was formerly confused with syphilis. It is characterized by the presence, on any part of the body, of more or less numerous warty fungoid lesions which tend to ulcerate. In this disease a primary lesion appears after a period of incubation and this is followed after another interval by a general eruption. It is not

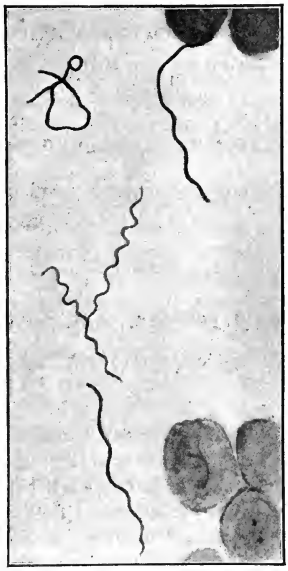


Fig. 183.—*Treponema pallidum* (in centre) and the *Spirocheta refringens*. (*Greene's Med. Diagnosis*)

a venereal disease as is the case with syphilis. It is caused by a slender spirochæte, *Treponema pertenue*, which is morphologically identical with the organism of syphilis. Animals which have been immunized to one of these diseases are found to be still susceptible to the other. The organisms in yaws are present in enormous numbers in the hypertrophied and swollen epidermis of the lesions whereas in syphilis they are confined to the deeper tissues. The disease responds more favorably even than syphilis to treatment with salvarsan which may

be thus regarded as practically a specific. It responds positively to the Wassermann test and is more uniform with respect to this test than syphilis.

Other Spirochætal Diseases

Ulceration granuloma of the pudenda is a tropical disease which has been claimed to be caused by a spirochæte, although its etiology still remains uncertain.

Spirochætes cause diseases of geese in southern Russia and of fowls in Brazil and in other tropical countries. The spirochæte of fowls, *Spirochæta gallinarum*, P. Blanchard, is transmitted by a tick, *Argas miniatus;* the means by which the goose spirochæte, *Spirochæta anserina*, Sacharoff, is carried is not known.

CHAPTER IV*

CONTROL OF INFECTIOUS DISEASES

Principles

That the infectious diseases can be controlled depends upon the facts that they arise only in the presence of a specific living infective agent; that they pass from patient to prospective patient only because the infective agent passes from patient to prospective patient; and that therefore the prevention of effective passage will prevent the spread of the disease. These preventive measures with their natural incidental developments constitute the practice of present public health relating to these diseases.

In general the infective agent leaves the body of the patient by the mucus-lined orifices of the body, the nose and the mouth, the anus, the urethra, the mammæ, and the genital organs. In general it must, if it is to infect successfully another person, reach one or more of the same mucus-lined orifices of that other person. Excluding the venereal diseases the ordinary infectious diseases (tuberculosis, typhoid fever, diphtheria, scarlet fever, measles, whooping cough, smallpox, chickenpox, pneumonic plague, leprosy) are received almost exclusively into the body through the mouth (or nose). While the passage is usually from mucous membrane to mucous membrane as above outlined, the infective agent may pass effectively from mucous membrane to cut or abrased skin (the uninjured skin is probably almost always resistant to these infections). Again, in those diseases where skin lesions are a prominent feature (smallpox, plague, leprosy) the infective agent may pass from the skin lesions to a mucous membrane, or to a cut or abrasion. But these are rare methods of transmission as compared with the mucous to mucous forms, except in syphilis and chancroid where they frequently occur.

The routes of travel between the patient and the prospective patient are many. At times, mucous membrane may be applied to

* Prepared by H. W. Hill.

mucous membrane as when a well person kisses a diphtheritic child; conveyance of particles through the air, sprayed from the mouth, may occur, as when a diphtheritic patient coughs into an attendant's face; or mucous membranes may be applied to skin or *vice versa*, as in the kissing of a smallpox patient; but in general the discharges are conveyed somewhat indirectly. The prime route from mucous membrane to mucous membrane is furnished by the hands. An attendant touches the patient's lip or wipes out the mouth or otherwise performs toilet services and receives the discharges upon his fingers. The fingers go then to the attendant's mouth directly, or touch something (the tines of a fork or the bowl of a spoon, etc.) which in turn goes into his mouth; or the attendant may touch the fork or spoon or food of others and thus they become infected. He may milk a cow and so get the discharges into the milk. With the infection in his own mouth he may kiss others and transfer it to them. It is impossible to outline the infinite combinations that may occur, but the principles are here made obvious. When the infective discharges handled are those of the bladder or bowel (as in typhoid fever, cholera, etc.) the same dangers of transmission are encountered and unfortunately too often realized. The wholesale discharge of sewage into water supplies is merely a gross example of the same principle of transfer of discharges from human bodies to the human mouth.

Another factor in the transmission of disease (as distinguished from the transmission of the germ) is the condition of the infectee. The germ is analogous to a seed; the methods of transmission are somewhat analogous to the distribution of seeds in nature; the condition of the infectee is analogous to the character and nutritional condition of the soil which the seed reaches.

If for any reason the germ will not develop in the soil where it is planted, or, still further, if it grows but fails to produce those poisons through which alone it acts, or finally if, growing and producing its poisons, the soil neutralizes the poisons, no disease results. Science, logic, and the law (each of which regards itself, and rightly so, as merely an apotheosis in its own line of "common sense") unite in the dictum that a disease exists only when the normal functions of the body are in some way interfered with to the detriment of the body. The mere infection of the body with a disease germ does not, in science, logic, or the law, constitute disease. Hence, the reception of a disease germ

into the body is but the first of three essentials, the other two being poison-production by the germ and poison-action on the tissues. Many persons are insusceptible to the poisons of one or more disease germs. In whatever way this insusceptibility originate, (natural, acquired by a previous attack, or acquired by artificial treatment) the existence of insusceptibilty tends to prevent the acquiring of the disease.

Practice

Undoubtedly, the one wholly efficient method of preventing the spread of infectious diseases would consist in immunizing all the possible infectees against all the possible diseases. Unfortunately, we know of no practical immunizing methods except in the case of a very few diseases, notably smallpox and typhoid fever, paratyphoid and cholera.

Our methods of control of any disease therefore begin with the attempt to destroy them at their origin in the body of the patient, but such methods are merely incidental to the destruction of the germs for the good of the patient himself, *i.e.*, they belong rather to therapeusis than to public health. Unfortunately, also, scarcely any efficient method of destroying bacteria within the body of the patient without destroying the patient also is known and therapeusis along this line contents itself largely as yet in so controlling the patient's condition as to permit and encourage to the highest the natural forces of the body to attack the germs. These natural forces, however, direct their chief energies and secure their chief results, not in destroying the germ but in neutralizing the poisons the germs throw off, and in practice, patients recover rather because they have neutralized the poisons than because they have killed or ejected the germs. For this reason a recovered patient often remains a breeding ground for the germs which caused the attack, but to whose poisons he is now resistant or immune.

Practically, then, the germs must leave the patient's body before they can be destroyed. It is at this stage that the most efficient control can be exercised, and that control consists in killing them before they become scattered. In practice the efficient disinfection of all the discharges of a patient will prevent the spread of any disease from him. But this is not as easy to do as at first might appear. Ridding the

body of its discharges in health is a process dependent on the individual, carried out by him consciously or unconsciously all his life by methods chiefly directed to conserve convenience rather than to prevent their spread. In health, the careless scattering of these discharges is not of great moment, but of course the habits of indifferent and careless discharge, acquired in health, persist after disease is contracted. The presence of an infective agent in the discharges renders the previously harmless scattering of the discharges the greatest menace that is known to the health of the associates. Hence one primary requisite in the personal warfare against the infectious diseases is to establish among all people such habits during health that even the normal discharges are not exchanged. This must be achieved by teaching the individual not to scatter his discharges and by teaching his associates not to receive them, if he does.

Accepting conditions as they are, the care of the sick by watchful, well-trained nurses who will prevent the spread of the discharges must largely take the place of the earlier training of the patient. Usually this also is impossible. It would seem that at least 95 per cent of the total cases of infectious disease in this country are cared for at home by the home folks, *i.e.*, untrained, worried, exhausted mothers chiefly, trying to learn in the actual face of the enemy, the technic and knowledge acquired quietly and systematically by the trained nurse. Hence, within the home, and at present, sanitary nursing to prevent spread of disease is a poor and often broken defence.

The third method of control is the destruction of the germs in passage from patient to prospective patient; and this must be largely confined to the actual discharges when accumulated in one place; the finer discharges thrown into the air can hardly be followed.

Under this head may be classed the disinfection of fæces and urine, the disinfection of bed clothing, eating utensils, etc., coming into contact with the patient, and especially the disinfection of the hands of attendants. The throats of attendants often contain the germ, especially when diphtheria, scarlet fever, measles, etc., are concerned. Unfortunately, the disinfection of the throat is extremely difficult and the scientific nurse will take every precaution to avoid receiving the germ into the mouth, rather than try to dislodge or destroy it after its reception. The use of a respirator is useful.

As outlined in the preceding section, the principles involved in con-

trolling infectious diseases are very simple, but in practice the individual cannot be trusted to avoid spreading his discharges, partly from ignorance, partly from carelessness, often from mere ingrained bad habits regarding the disposal of discharges, especially those of nose and mouth, indulged unconsciously by those who both know how and mean to be careful.

This would matter little were the infected persons always so sick as to be confined to the house or to bed, especially if during such confinement their discharges were under strict surveillance by scientific trained nurses.

But since many, perhaps half, of the infected persons are not sick enough (if sick at all) even to remain at home; since, also, even severe cases, under surveillance in bed during the height of the attack, have a prodromal stage and a convalescent stage during which they are going about although infective, it is not hard to see that the population of any community is likely to embrace at any time infective persons at large—persons who may or may not be aware of their own condition.

Theoretically and practically, then, the official control of infectious diseases must begin with the blanket assumption that the discharges of every individual must be confined to himself and especially prevented from reaching, through any public utility, the mouths of other citizens. Official control of the exchange of discharges by the individual within the family and in the absence of any specific proof that the discharges are infective, is impossible, although through various agencies the individual may be urged to that end. The moment, however, that the individual or the family engage in any occupation which permits them to inflict their discharges upon others, especially through food or milk, that moment should the individual or family come under official cognizance, their methods be inspected and their infectiveness estimated. The same arguments apply to aggregations of individuals from different families. So long as private meetings are held, it is difficult to supervise or prevent exchange of discharges. But public and especially compulsory meetings, at school, at church, at theatre, etc., should receive official attention. Provision should be made concerning all such meetings that they be held only in suitable places, without overcrowding. The exclusion by the officers, attendants, or the general public of all known to be infected or suspected of infection and of all who more openly disregard ordinary rules of decency in the

disposal of discharges (spitting, etc.), should be part of the duties of the health department.

Finally, the strictest supervision of those concerned publicly and officially in the handling of public utilities on a large scale (water supplies, milk supplies, hotels, restaurants, food stores, etc.) should hold all strictly accountable for the contamination of such supplies with discharges whether these be normal or not. Hence official control of infectious disease divides itself naturally as follows:

1. The recognition and isolation of frank cases of the diseases in question, at home or, better, in a proper hospital.

2. The supervision of the attendants and immediate associates of such frank cases.

(*a*) To detect among them that one from whom the frank case, already recognized, received his infection.

(*b*) To detect at the earliest moment any other frank case about to develop from among those associates who may have been infected at the same time and from the same source as the frank case already found.

(*c*) To prevent further spread from any already infected associates or those who may become infected by later association with the frank case during its existence as such.

3. The exclusion of the frank cases, their attendants and immediate associates, from participation in public life so long as danger continues and especially their exclusion from having to do with public utilities or public gatherings. Hence has arisen the crude drastic but efficient (when consistently and uniformly carried out in every case) system of isolation of the sick and quarantine of his associates.

Unfortunately quarantine has become a mere letter-of-the-law procedure, working great hardships on those who conscientiously submit to it and yet failing to achieve its objects because of the great number of those who evade or escape it; moreover, because its provisions are unintelligently enforced. Of what avail is rigid quarantine of an infected family where milk continues to be sold from the same premises? Why quarantine the honest man who has an honest physician and whose case is reported, while his neighbor, having the same disease in his family, calls no physician, or a dishonest one, and therefore escapes official cognizance?

The only remedy seems to be the recognition of the principle that harboring or having in possession a case of infectious disease, unknown

to the proper officials, is a crime against society, and that the excuse that the person harboring such case did not know it to be such should be of no more weight than the plea of ignorance of the law which is not allowed in other and often far less serious matters.

The official isolation of infectious cases involves also official responsibilities regarding the release from isolation after the acute attack is over. Officially to declare a person dangerous to the community does no harm to the community if a mistake is made. An official declaration that a person is no longer dangerous and is therefore free to enter into the community life again may, if mistaken, result in a widespread outbreak. No more delicate task confronts the public health official than the making of this decision.

In diphtheria, the examination of cultures from the throat and nose of the person in question and the repeated failure to find the bacterium of diphtheria is usually considered a safe criterion. In scarlet fever, complete and continued restoration of the throat and nose to normal conditions, together with absence of ear discharges, should be required, yet is not perfect; for it is not very unlikely that the scarlet fever infective agent, whatever it may be, can continue in a recovered scarlet fever throat as the diphtheria bacterium may remain in a recovered diphtheria throat. In other diseases the decision is based on similar lines—the disappearance of crusts in smallpox and chickenpox, of discharges in measles, on restoration to normal of whooping cough; but in all these diseases the analogy with diphtheria may hold to a greater or less extent. In tuberculosis, the patient is infective as long as *Bact. tuberculosis* can be found in the sputum; in typhoid fever the patient is likewise infective as long as the urine or fæces show the typhoid bacillus. In these two diseases, however, quarantine or even isolation is not officially carried out nor release from restriction officially given to any great extent or with any marked uniformity.

Full sanitary nursing precautions regarding a typhoid fever patient's discharges should continue for an average of three months after recovery.

Public Health Regulations are made for the good of the Public. They are as binding as your assistant with the prevention of disease. They are not made to interfere with the individual, more than is necessary as it may be dangerous to others.

I.—HOUSEHOLDERS RESPONSIBILITY TO THE BOARD OF HEALTH

Infectious Diseases:

It is required that whenever any householder knows or has reason to suspect that any member of the household has any communicable disease, he shall within twelve hours notify the Health Department.

Note.—The Medical Officer of Health ... may enter any house, etc. ... to suspect the presence of any ...

Births:

It is required that every birth ... shall be registered ... parent, guardian, ... householder ... form, within thirty days after the ... birth.

Deaths:

It is required that every death ... shall be registered ... within the death occurs in the province before it is buried ... ed

II.—PHYSICIANS RESPONSIBILITY TO THE BOARD OF HEALTH.

Infectious Diseases:

It is required that whenever any physician ... to visit is infected with any communicable disease, he shall within ... hours ... thereof.

Births:

It is required that every birth ... registered ... within thirty days ...

Note.—Further it must be the duty of every ... which he is in attendance is proper ... or Physician.

Deaths:

It is required by law that ... or Physician ... ceases, on the prescribed ... shall be issued.

Note.—Further it should be ... with the "International ...

III.—PENALTIES.

1.—Any person ... case of any communicable ... ever exceeding $100.00.

2.—Any person ... may incur a penalty not ... ing $50.

3.—Any person who wilfully ... ister required in regarding ... ith or for ... $50.

Comments on Table Showing Methods of Handling the Common Infectious Diseases, London, Canada

Experiment and experience indicate that certain changes are advisable, particularly in the length of isolation and in the currently accepted periods assigned as incubation periods. Up to date, the following changes have been made in the practice of the London (Can.) Health Department without evil results.

1. First line, fourth column, measles—change 3 weeks to 11 days for "clean" cases (*i.e.*, without sore throat, nose bleed, bad ears, etc.); but in cases showing sore throat, nose bleed, bad ears, etc., use 18 days.

2. No restrictions are now placed on *immunes* unless associating with actual patients, except in diphtheria (where immunity is not considered); and in scarlet fever (children excluded from school, if patient remains at home); and in smallpox which conforms to regulations for scarlet fever.

3. First line, seventh column, mumps—change 4 weeks to 3 weeks in uncomplicated cases. German measles change 3 weeks to 1 week in all cases.

4. Third column, scarlet fever—"after 5 days" is in practice interpreted as "on the sixth day after," and so in all other similar instances: thus measles, "after 14 days" means "on the fifteenth day after."

5. Non-immune contacts in typhoid should be observed to a date 21 days beyond last exposure instead of 14; in chickenpox for 18 days instead of 16; in mumps 25 days instead of 21; in German measles 16 days instead of 14.

6. Much time may be saved non-immune contacts by careful calculation of the really necessary period of observation: thus, illustrated, for measles (incubation period uniform, 10 days to prodromes; 4 days more to rash); and for mumps (incubation period variable, 14 days to 25 days to prodromes; ½ to 1 day more to enlargement of parotids, etc.).

Illustration: Measles. If a non-immune is exposed to measles July 1 to 4 inclusive, he may develop prodromes at any time between July 11 and 14 (inclusive), the rash following from July 15 to 18 (inclusive). Hence he may go about his business safely up to July 10; come under observation July 11 to July 18 inclusive, and if nothing has developed be released July 19.

Mumps: If a non-immune is exposed to mumps July 1 to 4, he may develop prodromes at any time from July 15 to July 29, the enlargement of the parotids appearing a half day to a day later. Hence he may go about his business safely up to July 14; come under observation to July 30; and if nothing has developed, be discharged July 31. Similar calculations may be made for the other diseases, always providing the period of exposure is definitely known.

Disinfection

Two systems of disinfection have been long recognized, *concurrent* and *terminal*. The former concerns the daily, hourly attention to, and disinfection of, everything coming in contact with the patient,

especially with his discharges and all that they may contaminate. The latter concerns the final disinfection of the patient's room, perhaps of the whole house, occupied by him during the attack, after the recovery of the patient.

Very much undue emphasis has been given to terminal disinfection. Large expenditures are made for this purpose and great faith placed in it, unfortunately to the exclusion of attention to, and reliance on, the infinitely more useful and logical concurrent disinfection, which, properly done, ought almost wholly to displace it.

Terminal disinfection should be done following tuberculosis of the lungs, anthrax and plague; in tuberculosis because of the great numbers and wide distribution of the bacteria thrown out by the patient, especially the careless patient; in anthrax because of the existence of resistant spores possibly attached to furniture, etc.; in plague because of the intense virulence of the organism and its tendency, like anthrax, to infect directly through the skin. In the ordinary diseases of the temperate zone, however, terminal disinfection cannot for a moment take the place of concurrent disinfection and is unnecessary if the former be properly carried out.

METHODS OF DISINFECTION

Concurrent Disinfection.—The disinfection of infected discharges, and of everything coming into contact with the discharges, whether the discharges be of the nose, mouth, bladder, or bowel, and whether the things which come into contact with the discharges be utensils, clothing, hands, furniture, etc., should be done at once, as soon as the discharges appear, or the articles, hands, etc., become contaminated.

Bladder and bowel discharges deposited directly in proper sewer-connected toilet-bowls require no disinfectant treatment; but the seat, door-knobs, toilet paper rack, flush pull and so on, which the discharges may reach, directly or through the patient's hands, should receive disinfection every time the toilet is used by such a patient. Where bed-pans or urinals are used and then emptied into such a toilet-bowl, disinfection should be done of the hands of the attendant who empties the pan, of the whole pan itself, and of any part of seat or bowl (not reached by the flush) contaminated by splash or dribbles from the bed-pan or urinal.

Where outdoor toilets or indoor toilets not connected with a sewer are in use the discharges must always be disinfected—preferably by half-filling the bed-pan or urinal, before use, with a saturated solution of milk of lime (unslaked lime, in water, to saturation—cool and pour off the liquid parts) into which the discharges are received. Where such toilets are used by the patient directly, an *abundant* layer of powdered unslacked lime should cover the discharges as soon as they are de-

posited. Such layer should be an inch deep. Precautions regarding the seats, door-knobs, hands, etc., should be followed as above described. The difficulty in enforcing these precautions makes fly-screening a better plan.

Soiled bed clothing or other clothing, handkerchiefs, etc., may be rolled up and placed directly in boiling water; but if some interval must elapse before they can be boiled, they should be put directly into 5 per cent carbolic acid solution, or 0.1 of 1 per cent bichloride of mercury solution or other disinfectant of similar killing power for at least half an hour. Thereafter they may be handled as uninfected clothing.

Eating utensils after use should go directly into boiling water for several minutes and then be washed in the ordinary way. Spoons used for medicine, toys, thermometers, etc., which it may be inconvenient or impossible to put into boiling water, should be immersed in 5 per cent carbolic acid or 0.1 per cent bichloride solution for half an hour, then washed.

These solutions may be used also for the hands and a large bowl of one or both of them (carefully labelled, and out of reach of children, etc.) should be constantly ready; into this the patient's and attendant's hands should be dipped after every contamination.

Discharges from the nose and mouth should be collected on paper or rags and burned at once. If inconvenient to burn them, they should be dropped into carbolic or bichloride solutions as above, and disposed of as harmless after a half-hour's soaking.

It is difficult to specify every form of contact to be guarded against by disinfection, but the foregoing are the chief ones to watch for, and the principles given should be widely and intelligently applied—remembering always that the *discharges* contain the *danger*.

Terminal Disinfection.—Sulphur disinfection (4 pounds burned for every 1,000 cubic feet of space, in the presence of steam sufficient to saturate the atmosphere) is effective for disease bacteria—also for roaches, bedbugs, etc., and for mice, rats, etc. But it injures fabrics by bleaching them, and metals by tarnishing them. Formaldehyde vapor is now used in its place for disinfection; but flies, bedbugs, etc., are not successfully exterminated thus. The most recent approved method for use in the disinfection of houses is the Minnesota State Board of Health potassium permanganate formaldehyde method.

For each 1,000 cubic feet of space the following should be used:

Potassium permanganate (crystals)	11 ounces
Solution formaldehyde (U. S. P. 1900)	11 ounces
Water	9 ounces

Directions for use:

Prepare the room to be disinfected by sealing all cracks, windows, ventilators, etc., and all the doors but the one for exit, with wet newspaper strips; open all blankets, drawers, etc.; separate and open up all books, clothing, etc., in the room. Have wet strips of paper in readiness to seal the last door when the disinfection has been started and the operator has left the room. The windows should be left unlatched so that they may be opened from the outside after the disinfection is completed.

Use a metal pail with lapped (not soldered) seams, or an earthenware receptacle, holding not less than fourteen (14) quarts, in which to mix the above ingredients. Place the receptacle on bricks standing in a pan of water, but the receptacle should not touch the water.

Place the 11 ounces of potassium permanganate in the receptacle, distributing it evenly over the bottom.

Mix the formaldehyde (11 ounces) and the water (9 ounces), and pour this mixture over the potassium permanganate in the receptacle.

This done, the operator should leave the room as quickly as possible, sealing the door behind him with the wet strips of paper prepared in advance for this purpose.

The directions above apply to the disinfection of a room containing 1,000 cubic feet or less. If a room contains more than 1,000 cubic feet of space, use one of the above disinfecting outfits for each 1,000 cubic feet or fraction thereof. Do not attempt to use a double charge in a container of even double capacity.

In disinfecting a whole house, begin with the most distant room and having mixed the potassium permanganate, formaldehyde, and water in the proper receptacle, close the door of the room and seal it at once as directed above. Proceed in this way in the disinfection of all the rooms. Leave the seals unbroken on the window and doors for six hours, after which the rooms should be opened up and thoroughly aired. The temperature of the room at the time of disinfection should not be below 70°F.

No paper, cotton, cloth, wood, or other combustible material should be in or near the disinfecting outfit for fear of fire, and no flame should be permitted in the room near the disinfecting outfit.

Carriage of Infection by Biological Agents

The transmission of yellow fever and malaria by mosquitoes, in the course of which the parasite causing the disease must undergo a whole series of developmental changes before the mosquito can become infective, is now well understood. But the mechanical carriage of infectious material by flies from privy vaults or bed pans or even mucous membranes or open wounds to food and drink or to other mucous membranes or wounds has not been very long established.

That typhoid fever and dysentery have many times occurred in epidemic form chiefly by the carriage of the infective agents by flies the writer firmly believes as the result of personal investigation, as well as from the reports of others. Similar mechanical carriage of infection on the outside of the body has been attributed to rats, dogs, cats, even to cows and horses. This must not be confused with the dissemination of certain diseases by horses actually sick with the disease (glanders) or

carrying the germs in their intestines (tetanus), by cows actually sick of tuberculosis, or by other similar instances of disease derived directly from preceding cases or carriers in the lower animals.

Another class of cases where lower animals convey disease by biting, and yet act merely mechanically is instanced by the septicæmia sometimes arising from bites of well animals (rats, snakes, mosquitoes, etc.), the bite acting merely to admit to the tissues pathogenic forms accidentally present in the animal's mouth or on the skin of the bitten person. These must be distinguished from cases where the animal transmits thus a disease from which it is itself suffering (as when a rabid dog spreads rabies by biting other animals or man) and from true poisoning by injection of animal products at the time of biting (as done by poisonous snakes, mosquitoes, etc.).

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