

*Saunders' Question Compend*s

UC-NRLF



5B 241 034

ESSENTIALS OF
BACTERIOLOGY
M. V. BALL, M. D.

Get the B

andard

A

MEI

RY

A New and
Dentistry
new and
of Bacill
Operatio
ment, et
Pocket I
full flexi

Surgery,
her with
ns, etc.;
Diseases,
f Treat-
American
ound in
D net.

JUST ISSUED
It con

WORDS
um

This book
tionary, and
finest quality,
and is just the
stant referenc

nts' dic-
er of the
e leather,
k for con-
ords, and

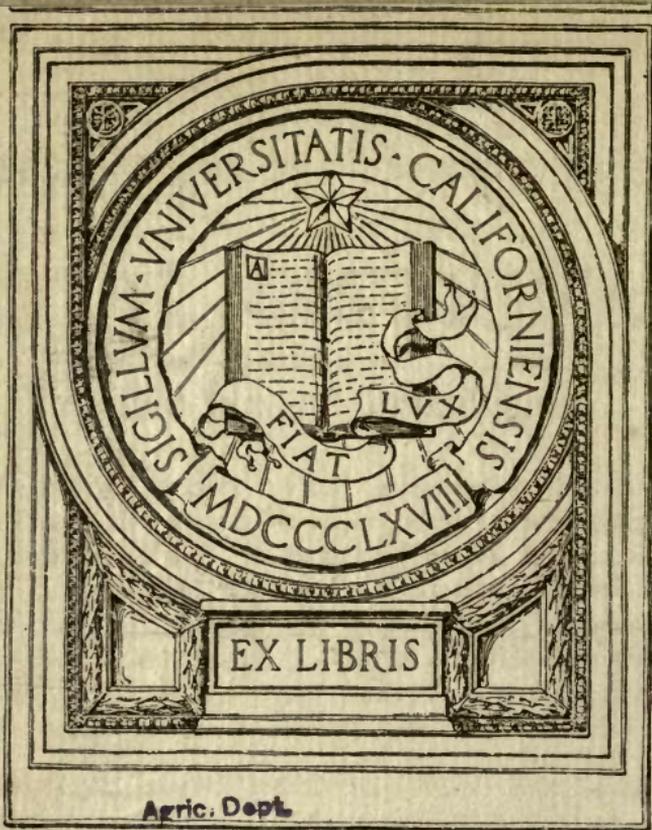
defines hundreds of important terms not to be found in any other dictionary. It is especially full in the matter of tables, containing more than a hundred of great practical value, including new tables of Tests, Stains and Staining Methods. A new feature is the inclusion of numerous handsome illustrations, many of them in colors, drawn and engraved specially for this book.

"I must acknowledge my astonishment at seeing how much he has condensed within relatively small space. I find nothing to criticise, very much to commend, and was interested in finding some of the new words which are not in other recent dictionaries."—ROSWELL PARK, *Professor of Principles and Practice of Surgery and Clinical Surgery, University of Buffalo.*

"Dr. Dorland's Dictionary is admirable. It is so well gotten up and of such convenient size. No errors have been found in my use of it."—HOWARD A. KELLY, *Professor of Gynecology, Johns Hopkins University, Baltimore.*

W. B. SAUNDERS COMPANY, 925 Walnut St., Phila.

London: 9, Henrietta Street, Covent Garden



Agric. Dept.

Main Lib.
Agric. Dept.

BIOLOGY
LIBRARY
G

Fifth Edition, Just Ready

With Complete Vocabulary

THE
AMERICAN POCKET
MEDICAL DICTIONARY

EDITED BY

W. A. NEWMAN DORLAND, A. M., M. D.,
Assistant Demonstrator of Obstetrics, University of Pennsylvania.

HUNDREDS OF NEW TERMS

Bound in Full Leather, Limp, with Gold Edges. Price, \$1.00 net;
with Patent Thumb Index, \$1.25 net.

The book is an **absolutely new one**. It is not a revision of any old work, but it has been written entirely anew and is constructed on lines that experience has shown to be the most practical for a work of this kind. It aims to be **complete**, and to that end contains practically all the terms of modern medicine. This makes an unusually large vocabulary. Besides the ordinary dictionary terms the book contains a wealth of **anatomical and other tables**. This matter is of particular value to students for memorizing in preparation for examination.

"I am struck at once with admiration at the compact size and attractive exterior. I can recommend it to our students without reserve."—JAMES W. HOLLAND, M. D., of *Jefferson Medical College*.

"This is a handy pocket dictionary, which is so full and complete that it puts to shame some of the more pretentious volumes."—*Journal of the American Medical Association*.

"We have consulted it for the meaning of many new and rare terms, and have not met with a disappointment. The definitions are exquisitely clear and concise. We have never found so much information in so small a space."—*Dublin Journal of Medical Science*.

"This is a handy little volume that, upon examination, seems fairly to fulfil the promise of its title, and to contain a vast amount of information in a very small space. . . . It is somewhat surprising that it contains so many of the rarer terms used in medicine."—*Bulletin Johns Hopkins Hospital, Baltimore*.

W. B. SAUNDERS COMPANY, 925 Walnut St., Phila.

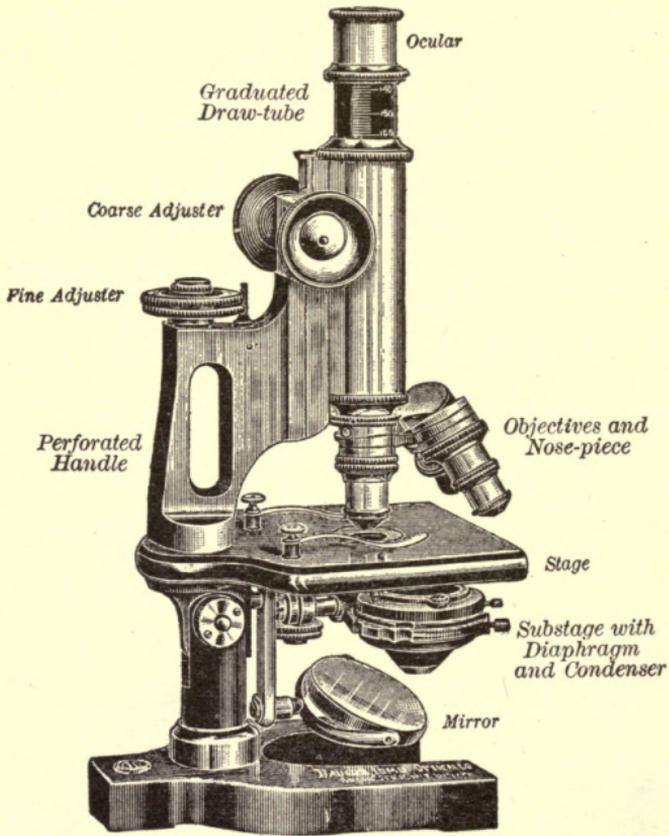
London: 9, Henrietta Street, Covent Garden

ESSENTIALS
OF
BACTERIOLOGY.

SINCE the issue of the first volume of the
Saunders Question-Compends,

OVER 275,000 COPIES

of these unrivalled publications have been sold.
This enormous sale is indisputable evidence
of the value of these self-helps to students
and physicians.



BACTERIOLOGIC MICROSCOPE.

SAUNDERS' QUESTION-COMPENDS, No. 20

ESSENTIALS

OF

BACTERIOLOGY

BEING A

CONCISE AND SYSTEMATIC INTRODUCTION TO THE
STUDY OF BACTERIA AND ALLIED MICROORGANISMS

BY

M. V. BALL, M.D.

MEMBER OF THE ACADEMY OF NATURAL SCIENCES OF PHILADELPHIA; CONSULTING
OPHTHALMOLOGIST AND AURIST TO THE STATE HOSPITAL AT WARREN, PA.;
WARREN COUNTY MEDICAL INSPECTOR FOR PENNSYLVANIA, DE-
PARTMENT OF HEALTH; FORMERLY INSTRUCTOR IN BAC-
TERIOLOGY AT THE PHILADELPHIA POLYCLINIC.

SIXTH EDITION, THOROUGHLY REVISED

With 135 Illustrations, some in Colors

PHILADELPHIA AND LONDON
W. B. SAUNDERS COMPANY

1908

QR46
B3
1908
BIOLOGY
LIBRARY
G

Main Lib.
Agric. Dept.

LIBRARY of CONGRESS
Two Copies Received
OCT 23 1908
Copyright Entry
Sep. 26, 1908
CLASS 217974 XXc. No.
COPY A.

Set up, electrotyped, printed, and copyrighted October, 1891. Reprinted October, 1892. Revised, reprinted, and recopyrighted May, 1893. Reprinted June, 1894. Revised, reprinted, and recopyrighted November, 1896. Reprinted October, 1898. Revised, reprinted, and recopyrighted March, 1900. Reprinted May, 1903. Revised, reprinted, and recopyrighted August, 1904. Reprinted October, 1905, and August, 1907. Revised, entirely reset, reprinted, and recopyrighted September, 1908.

Copyright, 1908, by W. B. Saunders Company.

LIBRARY OF CONGRESS
DUPLICATE
EXCHANGED

PRINTED IN AMERICA

PRESS OF
W. B. SAUNDERS COMPANY
PHILADELPHIA

M. N. W.

PREFACE TO THE SIXTH EDITION

THIS edition has been thoroughly revised and new chapters on Protozoa and Opsonic Technic added. While this work is intended as an introduction only, I have endeavored to make it complete enough to permit the student and the practitioner by its aid to examine and identify any or all of the important bacteria. It aims to contain the essentials of larger works and at the same time serve as a guide in the laboratory of the student, the clinician, and the sanatarian.

Medical bacteriology has gradually broadened its scope, so that now the accomplished bacteriologist must be well versed not only in a knowledge of allied microorganisms, but also in the knowledge of the higher life forms, the normal and pathologic anatomy of birds, mosquitoes, flies, rats, and fleas, through whom important diseases are transmitted to man. Although from the beginning bacteriology has been closely associated with zoölogy, never before has the subject required such an extensive knowledge of all biologic sciences, to say nothing of the depths in chemistry the studies of immunity have gradually entered. Elementary microbiology should be taught in close association with other sciences and, therefore, in a university as a preparatory course, while an advanced course only should be taught in medical schools in connection with pathology.

M. V. BALL.

WARREN, PA., *October, 1908.*

PREFACE TO THE FIRST EDITION

FEELING the need of a Compendium on the subject of this work, it has been our aim to produce a concise treatise upon the Practical Bacteriology of *to-day*, chiefly for the medical student, which he may use in his laboratory.

It is the result of experience gained in the Laboratory of the Hygienical Institute, Berlin, under the guidance of Koch and Fränkel; and of information gathered from the original works of other German, as well as of French, bacteriologists.

Theory and obsolete methods have been slightly touched upon. The scope of the work and want of space forbade adequate consideration of them. The exact measurements of bacteria have not been given. The same bacterium varies often much in size, owing to differences in the media, staining, etc.

We have received special help from the following books, which we recommend to students for further reference:

MACÉ: *Traité pratique de Bacteriologie.*

FRÄNKEL: *Grundriss der Bakterienkunde.*

EISENBERG: *Bakteriologische Diagnostik.*

CROOKSHANK, E. M.: *Manual of Bacteriology.*

GÜNTHER: *Einführung in das Studium der Bacteriologie, etc.*

WOODHEAD and HARE: *Pathological Mycology.*

SALMONSEN: *Bacteriological Technique (English translation).*

M. V. BALL.

CONTENTS

PART I

GENERAL CONSIDERATIONS AND TECHNIC

	PAGE
INTRODUCTION.....	15
CHAPTER I.—CLASSIFICATION, STRUCTURE, AND REPRO- DUCTION.....	17
“ II.—ORIGIN, LIFE, GROWTH, AND PROPERTIES..	22
“ III.—METHODS OF EXAMINATION.....	26
“ IV.—STAINING OF BACTERIA.....	30
“ V.—GENERAL METHOD OF STAINING SPECIMENS..	36
“ VI.—SPECIAL METHODS OF STAINING.....	40
“ VII.—METHODS OF CULTURE.....	44
“ VIII.—NUTRIENT MEDIA.....	49
“ IX.—SOLID TRANSPARENT MEDIA.....	53
“ X.—INOCULATION OF GELATIN AND AGAR.....	62
“ XI.—GROWTH AND APPEARANCES OF COLONIES..	68
“ XII.—CULTIVATION OF ANAËROBIC BACTERIA.....	71
“ XIII.—INFECTION.....	75
“ XIV.—IMMUNITY.....	78
“ XV.—ANIMAL EXPERIMENTS.....	84
“ XVI.—OPSONIC TECHNIC.....	87

PART II

SPECIAL BACTERIOLOGY

CHAPTER XVII.—NON-PATHOGENIC BACTERIA.....	91
<i>Bacillus Prodigiosus</i>	91
<i>Indicus</i>	92

	PAGE
Bacillus Mesentericus Vulgatus.....	92
Megaterium.....	93
Ramosus.....	93
Bacterium Zopfi.....	94
Bacillus Subtilis.....	94
Spinosus.....	95
Some Bacteria in Milk.....	95
Bacillus Acidi Lactici.....	95
Boas-Oppler.....	96
Butyricus.....	96
Amylobacter.....	97
Lactis Cyanogenus.....	97
Erythrogenes.....	98
Examination of Milk in Stained Specimens.....	98
Some Non-Pathogenic Bacteria of Water.....	99
Bacillus Violaceus.....	99
Cœruleus.....	99
Fluorescent Bacteria.....	99
Phosphorescent Bacteria.....	100
Leptothrix, Crenothrix, Cladothrix, and Beggiatoa.....	101
Microorganisms found in Urine.....	102
Spirillum.....	103
Rubrum; Concentricum.....	103
Sarcina.....	104
Lutea.....	104
Aurantica Flava, Rosea, and Alba.....	105
Ventriculi.....	105
CHAPTER XVIII.—PATHOGENIC BACTERIA.....	105
Bacteria Pathogenic for Man and Other Animals....	105
Bacillus Anthracis.....	105
Tuberculosis.....	109
Lepra Bacillus.....	120

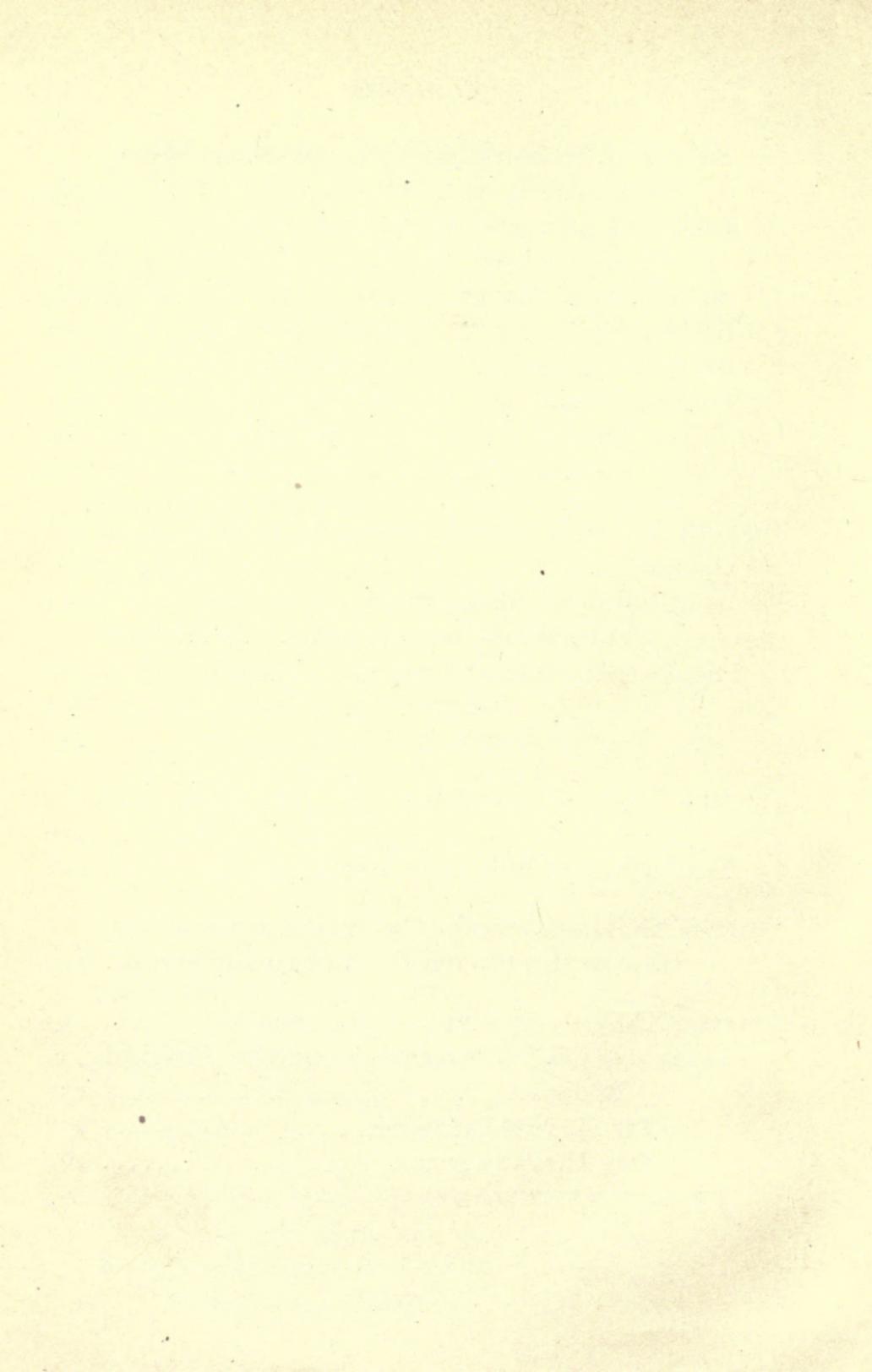
	PAGE
Syphilis Bacillus.....	120
Bacillus of Glanders.....	121
Mallein.....	123
Bacillus of Diphtheria.....	123
of Typhoid Fever.....	129
Paracolon or Paratyphoid.....	136
Psittacosis.....	137
Coli Communis.....	137
 CHAPTER XIX.—PATHOGENIC BACTERIA—CONTINUED...	 140
Spirillum Cholerae.....	140
Bacteria Similar to Spirillum Cholerae.....	146
Finkler-Prior.....	146
Tyrogenum.....	147
Vibrio Metschnikovi.....	147
Bacteria of Pneumonia.....	148
Pneumobacillus of Friedländer.....	150
of Fränkel.....	151
Antitoxin of Pneumonia.....	153
Bacillus of Rhinoscleroma.....	153
Diplococcus Intracellularis Meningitidis.....	153
Micrococcus Tetragenus.....	155
Capsule Bacillus.....	156
Bacillus of Influenza.....	157
Microorganisms of Suppuration.....	158
Streptococcus Pyogenes.....	159
Staphylococcus Pyogenes Aureus.....	160
Pyogenes Albus.....	162
Micrococcus Pyogenes Citreus.....	162
Cereus Albus.....	162
Flavus.....	162
Pyogenes Tenuis.....	162
Bacillus Pyocyaneus.....	163
Koch-Weeks' Bacillus.....	165

	PAGE
Morax-Axenfeld Diplobacillus of Conjunctivitis.	166
Micrococcus Gonorrhœæ.	166
Microbes Similar to Gonorrhœa	168
Protective Vaccines in Pus Infections.	170
Bacillus of Tetanus.	170
Œdematis Maligni.	174
of Soft Chancre.	177
of Bubonic Plague.	177
of Dysentery.	179
Aërogenes Capsulatus.	180
Micrococcus Melitensis.	181
Bacillus Enteritidis Sporogenes.	182
Protozoa.	183
Life Cycle of the Malarial Sporozoa.	184
Three Forms of Malarial Protozoa.	187
Methods of Examination for Malarial Organisms.	189
Trypanosoma	190
Lewisi.	191
Brucei.	191
Ugandense Gambiense.	192
Evansi.	192
Piroplasma Bovis.	192
Negri Bodies.	193
Spirillum of Relapsing Fever.	193
Spirochæte Pallida.	194
Amœba Dysenteriæ.	195
Small-pox and Vaccinia.	196
Yellow Fever.	196

CHAPTER XX.—BACTERIA PATHOGENIC FOR ANIMALS, BUT

NOT FOR MAN.	196
Bacillus of Symptomatic Anthrax.	196
of Chicken Cholera.	198

	PAGE
Bacteria of Hemorrhagic Septicemia, Swine Plague, Duck Cholera, etc.....	200
Bacillus of Erysipelas of Swine.....	201
Murisepticus.....	202
Micrococcus of Mal de Pis.....	203
Bacillus Alvei.....	204
Bacterium Termo.....	205
Proteus Vulgaris.....	205
Mirabilis.....	205
Zenkeri.....	205
CHAPTER XXI.—YEASTS AND MOULDS.....	206
Oïdiums.....	207
Cladotrices and Streptotrices.....	212
Streptothrix or Cladotrix Actinomyces (Ray-fungus).....	212
Maduræ.....	214
Farcinica.....	215
CHAPTER XXII.—EXAMINATION OF AIR, SOIL, AND WATER.....	216
The Bacteria of Milk and Other Foods.....	228
CHAPTER XXIII.—BACTERIOLOGIC EXAMINATION OF THE ORGANS AND CAVITIES OF THE HUMAN BODY..	229
CHAPTER XXIV.—ANTISEPTICS AND ANTISEPSIS.....	233
Tables of Chief Characteristics of the Principal Bacteria.....	238
Part I. Non-Pathogenic.....	238
Part II. Pathogenic.....	262



ESSENTIALS OF BACTERIOLOGY

INTRODUCTION

History.—The microscope was invented about the latter part of the sixteenth century, and soon after, by its aid, minute organisms were found in decomposing substances. Kircher, in 1646, suggested that diseases might be due to similar organisms, but the means at his disposal were insufficient to enable him to prove his theories. Anthony Van Leeuwenhoek, of Delft, Holland (1680 to 1723), so improved the instrument that he was enabled thereby to discover microorganisms in vegetable infusion, saliva, fecal matter, and scrapings from the teeth. He distinguished several varieties, showed them to have the power of locomotion, and compared them in size with various grains of definite measurement. It was a great service that this “Dutch naturalist” rendered the world; and he can rightly be called the “father of microscopy.”

Various theories were then formulated by physicians to connect the origin of different diseases with bacteria; but no proofs of the connection could be obtained. Andry, in 1701, called bacteria *worms*. Müller, of Copenhagen, in 1786, made a classification composed of two main divisions—*monas* and *vibrio*; and with the aid of the compound microscope was better able to describe them. Ehrenberg, in 1833, with still better instruments, divided bacteria into four orders: *bacterium*, *vibrio*, *spirillum*, and *spirochæte*. It was not until

1863 that any positive advance was made in connecting bacteria with disease. Rayer and Davaine had, in 1850, already found a rod-shaped bacterium in the blood of animals suffering from *splenic fever* (*sang de rate*), but they attached no special significance to their discovery until Pasteur made public his grand researches in regard to fermentation and the rôle bacteria played in the economy. Then Davaine resumed his studies, and in 1863 established by experiments the bacterial nature of splenic fever or anthrax.

But the first complete study of a contagious affection was made by Pasteur in 1869, in the diseases affecting silk-worms,—*pébrine* and *flacherie*,—which he showed to be due to micro-organisms.

Then Koch, in 1875, described more fully the anthrax bacillus, gave a description of its spores and the properties of the same, and was enabled to cultivate the germ on artificial media; and, to complete the chain of evidence, Pasteur and his pupils supplied the last link by reproducing the same disease in animals by artificial inoculation from pure cultures. The study of the bacterial nature of anthrax has been the basis of our knowledge of all contagious maladies, and most advances have been made first with the bacterium of that disease.

Since then bacteriology has grown to huge proportions—become a science in itself—and thousands of earnest workers are adding yearly solid blocks of fact to the structure, which structure it will be our aim briefly to describe in the pages which are to follow.

PART I

GENERAL CONSIDERATIONS

CHAPTER I

BACTERIA

THE bacteria occupy the lowest plane of plant life known to us, though they are by no means as primitive in their biology as was formerly supposed, and it is quite possible that still simpler forms may be discovered.

The numerous unicellular vegetable organisms which form the lower limit of plant life as we know it multiply by fission and are hence called the *Schizophyta*, or splitting plants. This group is subdivided into two classes—(a) the *Schizophyceæ*, or

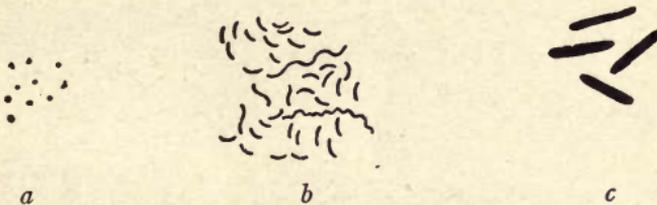


Fig. 1.—Types of bacteria: a, Micrococcus; b, spirillum; c, bacillus.

fission algæ, and (b) the *Schizomycetes*, or fission fungi, or bacteria, as we usually call them.

Bacteria are unicellular masses of protoplasm of microscopic size, multiplying by fission and existing without chlorophyl. Three main types are found: (1) Globular forms, called cocci; (2) straight rod-shaped forms, called bacilli; (3) curved or spiral rods, called spirilla. (See Fig. 1.)

Structure.—Bacteria are cells; they appear as round or cylindrical, of an average diameter on transverse section of 0.001

mm. (= 1 micromillimeter), written 1 μ . The cell, as other plant-cells, is composed of a membranous cell-wall and cell-contents; nuclei are not found.

Cell-wall.—The cell-wall is composed either of plant cellulose, or a form of albumin, since it is less permeable than cellulose membrane. The membrane is firm, and can be brought plainly into view by the action of iodine upon the cell-contents, which contract them.

Cell-contents.—The contents of the cell consist mainly of protoplasm, usually homogeneous, but in some varieties finely

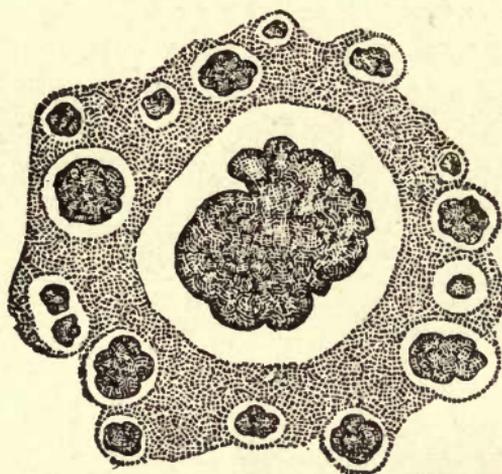


Fig. 2.—Zoöglea.

granular, or holding pigment, chlorophyll, fat-droplets, and sulphur in its structure. It permits osmosis, and is like other plant-cells.

Gelatinous Membrane.—The outer layer of the cell-membrane can absorb water and become gelatinoid, forming either a little envelop or capsule around the bacterium or preventing the separation of the newly branched germs, forming chains and bunches, as *streptococci* and *staphylococci*. Long filaments are also formed.

Zoöglea.—When this gelatinous membrane is very thick, irregular masses of bacteria will be formed, the whole growth

being in one jelly-like lump. This is termed a zoöglea ($\zeta\omega\acute{o}\nu$, animal, $\chi\lambda\omicron\iota\acute{o}\varsigma$, glue) (Fig. 2).

Locomotion.—Many bacteria possess the faculty of self-movement, carrying themselves in all manner of ways across the microscopic field—some very quickly, others leisurely.

Vibratory Movements.—Some bacteria vibrate in themselves, appearing to move, but they do not change their place; these movements are denoted as molecular or “*Brownian*,” and are due to purely physical causes.

Flagella.—Little threads or lashes are found attached to many of the motile bacteria, either at the poles or along the

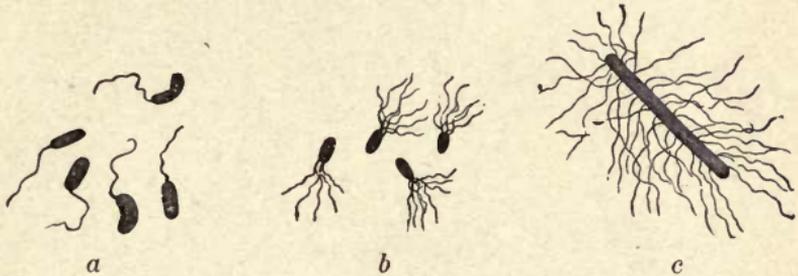


Fig. 3.—Types of flagella: *a*, *Vibrio cholerae*, one flagellum at the end—monotrichia type; *b*, *Bacterium syncyaneum*, tuft of flagella at the end, rarely at the side—lophotrichia type; *c*, *Bacterium vulgare*, flagella arranged all about—peritrichia type (Lehmann and Neumann).

sides—sometimes only one, and on some several, forming a tuft.

These flagella are in constant motion, and can probably be considered as the organs of locomotion; they have not been discovered upon all the motile bacteria, owing, no doubt, to our imperfect methods of observation. They can be stained and have been photographed. (See Fig. 3.) Flagella serve sometimes to increase food-supply, and have been found on some species which are non-motile.

Reproduction.—Bacteria multiply through simple *division* or fission, as it is called. Spore formation is simply a resting stage and not a means of multiplication. To accomplish division the cell elongates, and at one portion, usually the middle, the cell-wall indents itself gradually, forming a septum

and dividing the cell into two equal parts, just as occurs in the higher plant and animal cells. (See Fig. 4.)

Successive divisions take place, the new members either existing as separate cells or forming part of a community or group. It has been computed that if division takes place every hour, as it often does, one individual in twenty-four hours will have 17,000,000 descendants.

Spore Formations.—Two forms of sporulation, *endosporous* and *arthrosporous*.

Endosporous.—First, a small granule develops in the protoplasm of a bacterium; this increases in size, or several little

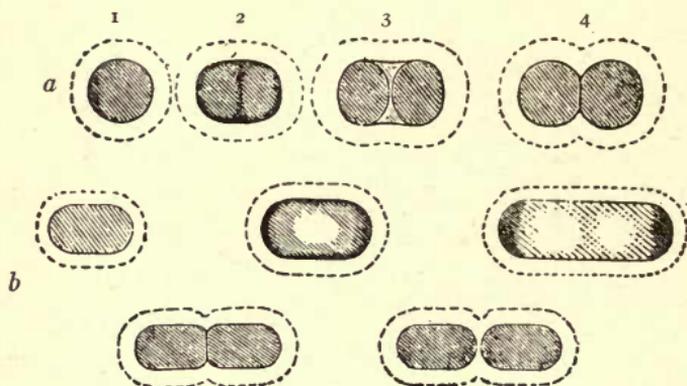


Fig. 4.—Division of bacteria: *a*, Division of a micrococcus; *b*, division of a bacillus (after Macé).

granules coalesce to form an elongated, highly refractive, clearly defined object, rapidly attaining its real size, and this is the spore. The remainder of the cell-contents has now disappeared, leaving the spore in a dark, very resistant membrane or capsule, and beyond this the weak cell-wall. The cell-wall dissolves gradually or stretches and allows the spore to be set free.

Each bacterium gives rise to but one spore. It may be at either end or in the middle (Fig. 5). Some rods take on a peculiar shape at the site of the spore, making the rod look like a drum-stick or spindle—clostridium (Fig. 6).

Spore Contents.—What the real contents of spores are is not

known. In the mother-cell at the site of the spore little granules have been found which stain differently from the rest of the cell, and these are supposed to be the beginnings—the *sporogenic bodies*. The most important part of the spore is its *capsule*; to this it owes its resisting properties. It consists of two separate layers—a thin membrane around the cell, and a firm outer gelatinous envelop.

Germination.—When brought into favorable conditions, the spore begins to lose its shining appearance, the outer firm membrane begins to swell, and it now assumes the shape and size

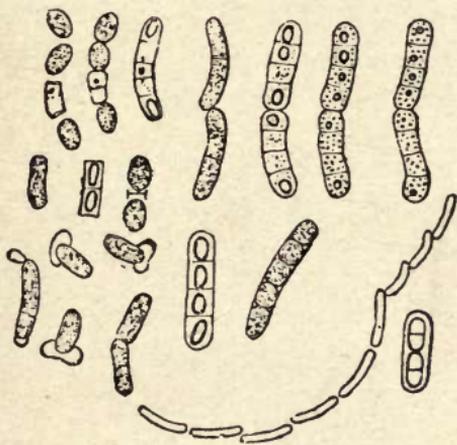


Fig. 5.—Sporulation (after De Bary).

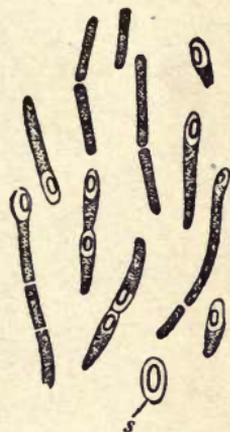


Fig. 6.—Clostridium.

of the cell from which it sprang, the capsule having burst, so as to allow the young bacillus to be set free.

Requisites for Spore Formation.—It was formerly thought that when the substratum could no longer maintain it, or had become infiltrated with detrimental products, the bacterium-cell produced spores, or rather turned itself into a spore to escape annihilation; but we believe now that only when conditions are the most favorable to the well-being of the cell, does it produce fruit, just as with every other type of plant or animal life, a certain amount of oxygen and heat being necessary for good spore formation. The question is still unsettled, however.

Asporogenic Bacteria.—Bacteria can be so damaged that they will remain sterile—not produce any spores. This condition can be temporary only or permanent.

Arthrosporous.—In the other group, called arthrospores, individual members of a colony or aggregation leave the same, and become the originators of new colonies, thus assuming the character of spores.

The micrococci furnish examples of this form.

Some authorities have denied the existence of the arthrosporous formation.

Resistance of Spores.—Because of the very tenacious envelop, the spore is not easily influenced by external measures. It is said to be the most resisting object of the organic world.

Chemical and physical agents that easily destroy other life have very little effect upon it.

Many spores require a temperature of 140° C. dry heat for several hours to destroy them. The spores of a variety of potato bacillus (*Bacillus mesentericus*) can withstand the application of steam at 100° C. for four hours.

CHAPTER II

ORIGIN OF BACTERIA AND THEIR DISTRIBUTION

AS Pasteur has shown, all bacteria develop from preëxisting bacteria or the spores of the same. They cannot arise out of nothing.

The wide and almost universal diffusion of bacteria is due to the minuteness of the cells and the few requirements for their existence. In a drop of water 1,700,000,000 cocci can find space.

Very few places are free from germs; the air on the high seas and on the mountain-tops is said to be free from bacteria, but this is questionable.

One kind of bacterium will not produce another kind. A bacillus does not arise from a micrococcus, or the typhoid fever bacillus produce the bacillus of tetanus.

This subject has been long and well discussed, and it would take many pages to state the "pros" and "cons"; therefore this positive statement is made, it being the position now held by the principal authorities.

Saprophytes and Parasites (*Saprophytes*: σαπρός, putrid; φυτόν, plant. *Parasites*: παρά, aside of; σίτος, food).—Those bacteria which live on the dead remains of organic life are known as saprophytic bacteria, and those which choose the living bodies of their fellow-creatures for their habitat are called parasitic bacteria. Some, however, develop equally well as saprophytes and parasites. They are called *facultative parasites*.

Conditions of Life and Growth of Bacteria.—*Influence of Temperature.*—In general, a temperature ranging from 10° C. to 40° C. is necessary to their life and growth.

Saprophytes take the lower temperatures; parasites, the temperature more nearly approaching the animal heat of the warm blooded. Some forms require a nearly constant heat, growing within very small limits, as the bacillus of tuberculosis.

Some forms can be arrested in their development by a warmer or colder temperature, and then restored to activity by a return to the natural heat.

A few varieties exist only at freezing-point of water, and others again will not live under a temperature of 60° C.

For the majority of bacteria a temperature of 60° C. is destructive; and several times freezing and thawing very fatal.

Influence of Oxygen.—Two varieties of bacteria in relation to oxygen—the one *aërobic*, growing in air; the other, *anaërobic*, living without air.

Obligate aërobins, those which exist only when oxygen is present.

Facultative aërobins, those that live best when oxygen is present, but can live without it.

Obligate or true anaërobins, those which cannot exist where

oxygen is; *facultative anaërobins*, those which exist better where there is no oxygen, but can live in its presence.

Some derive the oxygen which they require out of their nutriment, so that a bacterium may be aërobie and yet not require the presence of free oxygen.

Aërobins may consume the free oxygen of a region and thus allow the anaërobins to develop. By improved methods of culture many varieties of anaërobins have been discovered.

Influence of Light.—Sunlight is very destructive to bacteria. A few hours' exposure to the sun has been fatal to anthrax bacilli and the cultures of *Bacillus tuberculosis*. The sun's rays, however, must come in direct contact with the germs, and are usually active only on the surface cultures. The rays at the violet end of the spectrum are the most active. The electric arc-light has much the same effect as sunlight on bacteria.

Effects of Electricity.—Electricity arrests growth.

Effects of Röntgen Rays.—Have little or no effect on artificial cultures, but in the living tissues a pronounced bactericidal effect is produced, perhaps through the stimulation of the body-cells.

Vital Actions of Microbes.—Bacteria feeding upon organic compounds produce chemical changes in them, not only by the withdrawal of certain elements, but also by the excretion of these elements changed by digestion. Sometimes such changes are destructive to themselves, as when lactic and butyric acids are formed in the media.

Oxidation and reduction are carried on by some bacteria. Ammonia, hydrogen sulphid, and trimethylamin are a few of the chemical products produced by bacteria. Nitrites in the soil are reduced to ammonia.

Nitrification.—Albuminoids changed into indol, skatol, leucin, etc.; then these into ammonia, ammonia into nitrites, nitrites into nitrates.

Ptomains.—Brieger found a number of complex alkaloids closely resembling those found in ordinary plants, and which he named ptomains, from $\pi\tau\tilde{\omega}\mu\alpha$, corpse, because obtained from putrefying objects.

Proteins.—The *proteid contents* of the bacterial cell may cause inflammation and fever.

Putrefaction.—When fermentation is accompanied by development of offensive gases, a decomposition occurs, which is called putrefaction, and this, in organic substances, is due entirely to bacteria.

Producers of Disease.—Various pathologic processes are caused by bacteria, the name given to such diseases being *infectious diseases*, and the germs themselves called disease-producing or *pathogenic bacteria*. Those which do not form any pathologic process are called *non-pathogenic bacteria*.

Ferments may be *diastatic*, changing starch into sugar, or *proteolytic*, transforming albumins into more soluble substances, of which gelatin liquefaction is an example. *Inverting*, changing a sugar from one that does not undergo fermentation into one that does.

Coagulating, fat-splitting, hydrolytic ferments are some of the other varieties.

Toxins and toxalbumins are various albuminoids produced in the animal organism and in culture-media which are very poisonous, and are considered the prime cause of disease.

Pigmentation.—Some bacteria are endowed with the property of forming pigments either in themselves, or producing a chromogenic body which, when set free, gives rise to the pigment. In some cases the pigments have been isolated and many of the properties of the anilin dyes discovered in them.

Phosphorescence.—Many bacteria have the power to form light, giving to various objects which they inhabit a characteristic glow or phosphorescence.

Fluorescence.—An iridescence, or play of colors, develops in some of the bacterial cultures.

Gas-formation.—Many bacteria, anaërobic ones especially, produce gases, noxious and odorless; in the culture-media the bubbles which arise soon displace the media.

Odors.—Some germs form odors characteristic of them: some are pleasant and even fragrant; others, foul and nauseous.

Effect of Age.—With age, bacteria lose their strength and die.

CHAPTER III

METHODS OF EXAMINATION

WE divide the further study of the general characteristics of bacteria into two portions:

First, the examination of bacteria by aid of the microscope.

Second, the continued study through artificial cultivation.

They both go hand in hand; the one incomplete without the other.

Microscopic.—The ordinary microscope will not suffice for bacteriologic research. Certain special appliances must first be added. It is not so much required to have a picture very large, as to have it sharp and clear.

Oil-immersion Lens.—The penetration and clearness of a lens are very much influenced by the absorption of the rays of light emerging from the picture. In the ordinary dry system many of the light rays, being bent outward by the air which is between the object and the lens, do not enter the lens, and are lost. By interposing an agent which has the same refractive index as glass, *cedar-oil* or *clove-oil*, for example, all the rays of light from the object enter directly into the lens.

The "homogeneous system," or oil-immersion lens, consists of a system of lenses which can be dipped into a drop of cedar-oil placed upon the cover-glass, and which is then ready for use.

Abbé's Condenser.—The second necessary adjunct is a combination of lenses placed underneath the stage, for bringing wide rays of light directly under the object. It serves to intensify the colored pictures by absorbing or hiding the unstained structure.

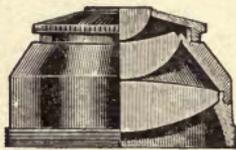


Fig. 7.—Abbé's condenser.

This is very useful in searching a specimen for bacteria, since it clears the field of everything that is not stained. It is called Abbé's condenser (Fig. 7). Together with it is usually found an instrument

for shutting off part of the light—a *blender* or diaphragm (Fig. 8). When the bacteria have been found, and their relation to the structure is to be studied, the “Abbé” is generally shut out by the iris blender, and the structure comes more plainly into view. A white light (daylight or a Welsbach burner) is best for bacterial study: use the plane mirror for daylight and the concave mirror for artificial light.

For all *stained bacteria* the oil-immersion lens and Abbé condenser, without the use of blender. For *unstained specimens*, oil-immersion and the narrowed blender.

When examining with low-power objective, use a *strong* ocular. When using high-power objective use *weak* ocular. A

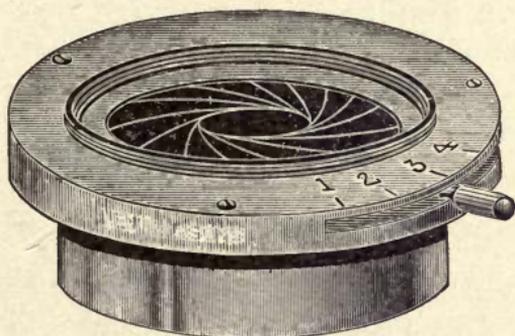


Fig. 8.—Iris blender

revolving nose-piece will be found very useful, since it is sometimes necessary to change the objective on the same field, and this insures a great steadiness of the object.

Great cleanliness is needed in all bacteriologic methods, but nowhere more so than in the microscopic examination.

The cover-glass should be very carefully washed in alcohol, and dried with a soft linen rag. To remove the stains on the cover-glasses that have been used, they should be soaked in hydrochloric acid or placed in a 6 per cent. aqueous solution of potassium dichromate with 6 per cent. of strong sulphuric acid, washed in water, and kept in absolute alcohol.

Examination of Unstained Bacteria.—As the coloring of bacteria kills them and changes their shape to some extent, it

is preferable to examine bacteria, when possible, in their natural state.

We obtain the bacteria for examination either from liquid or solid media.

From Liquids.—With a long platinum needle the end of which is bent into a loop (Fig. 9, *a*) obtain a small drop from the liquid containing the bacteria, and place it on a cover-glass or slide, careful that no bubbles remain.

Sterilize Instruments.—Right here we might say that it is best to accustom one's self to pass all instruments, needles, etc., through the flame before and after each procedure; it insures safety; and once in the habit, it will be done automatically.

From Solid Media.—With a straight-pointed platinum needle (Fig. 9, *b*) a small speck of the medium is taken and

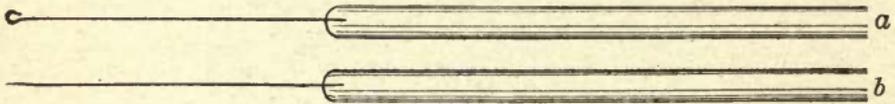


Fig. 9.—Platinum needles for transferring bacteria made from No. 27 platinum wire inserted in glass rods: *a*, Looped needle; *b*, straight-pointed needle (McFarland)

rubbed upon a glass slide with a drop of sterilized water or bouillon, and from this a little is taken on cover-glass, as before.

The cover-glass with its drop is now placed on the glass slide, carefully pressing out all bubbles. Then a drop of cedar-oil is laid on top of the cover-glass, and the oil-immersion lens dipped gently down into it as close as possible to the cover-glass, the narrow blender *shutting off* the Abbé condenser, for this being an unstained specimen, we want but *little light*. We now apply the eye, and if not in focus, use the fine adjustment or the coarse, but always *away from the object*—*i. e.*, toward us—since the distance between the specimen and the lens is very slight, it does not require much turning to break the cover-glass and ruin the specimen. Having found the bacterium, we see whether it is bacillus, micrococcus, or spirillum; discover if it is motile or not. That is about all we can ascertain by this method.

Hanging Drop (Fig. 10).—When the looped platinum needle is dipped into a liquid, a very finely formed globule will hang to it; this can be brought into a little cupped glass slide (an ordinary microscopic glass slide with a circular depression in the center) in the following manner: The drop is first brought upon a cover-glass; the edges of the concavity on the glass slide are smeared with vaselin, and the slide inverted over the drop; the cover-glass sticks to the smeared slide, which, when turned over, holds the drop in the depression covered by the cover-glass, thus forming an air-tight cell; here the drop cannot evaporate. Both slide and cover-glass should first be sterilized by heat.

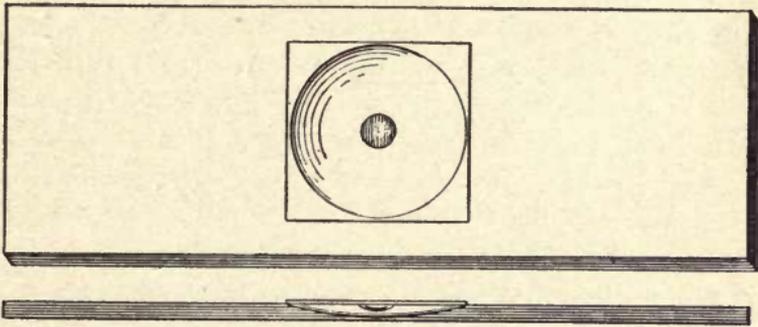


Fig. 10.—A “concave slide” with “hanging drop” (McFarland).

Search for the bacteria with a weak lens; having found them, place a drop of cedar-oil upon the cover-glass, and bring the oil immersion into place (here is where a nose-piece comes in very useful), careful not to press against the cell, for the cover-glasses are very fragile in this position.

Search the *edges* of the drop rather than the middle; the bacteria will usually be very thick in the center and not so easily distinguished.

Spores, automatic movements, fission, and cultivation in general can be studied for several days. This *moist chamber* can be placed in a brood-oven or on the ordinary warming stages of the microscope.

Agglutination as observed in Widal’s test is best seen in the hanging drop.

CHAPTER IV

STAINING OF BACTERIA

STAINING or coloring bacteria is done in order to make them prominent and to obtain permanent specimens. It is also necessary to bring out the structure of the bacteria, and serves in many instances as a means of diagnosis; it would be well-nigh impossible to discover them in the tissues without staining.

Anilin Colors.—Of the numerous dyes in the market, nearly all have, at one time or other, been used in staining bacteria. But now only a very few find general use, and with methylene-blue and fuchsin nearly every object can be accomplished.

Basic and Acid Dyes.—Ehrlich was the first to divide the anilin dyes into two groups, the basic colors to which belong—

Gentian-violet, or pyoktanin.

Basic fuchsin.

Methyl-violet, or dahlia.

Bismarck-brown.

Methylene-blue (*not* methyl blue).

Thionin.

Safranin.

And the acid colors to which *eosin* and acid fuchsin belong.

The *basic* dyes stain the bacteria and the nuclei of cells; the *acid* dyes stain chiefly the tissue, leaving the bacteria almost untouched. *Carmin* and *hematoxylin* are also useful as contrast stains, affecting bacteria very slightly. The anilin dyes are soluble in alcohol or water or a mixture of the two.

Staining Solutions.—A saturated solution of the dye is made with alcohol. This is called the *stock* or *concentrated* solution; 1 part of this solution to about 100 parts of distilled water constitutes the ordinary aqueous solution in use or *weak* solution.

It is readily made by adding to an ounce bottle of distilled water enough of the strong solution until the fluid is still opaque in the body of the bottle, but clear in the neck of the same.

These weak solutions should be *renewed* every three or four weeks, otherwise the precipitates formed will interfere with the staining.

Compound Solutions.—By means of certain chemical agents the intensity of the anilin dyes can be greatly increased.

Mordants.—Agents that “bite” into the specimen, carrying the stain with them, depositing it in the deeper layers, are called mordants or etchers.

Various metallic salts and vegetable acids are used for such purpose.

The mother liquid of the anilin dyes, *anilin-oil*, a member of the aromatic benzol group, has also this property.

Anilin-oil Water.—Anilin-oil is shaken up with water and then filtered; the anilin water so obtained is mixed with the dyes, forming the “anilin-water gentian-violet” or anilin-water fuchsin, etc.

Carbol-fuchsin.—Carbolic acid or phenol can be used instead of anilin-oil, and forms one of the main ingredients of Ziehl’s or Neelsen’s solution, used principally in staining *Bacillus tuberculosis*. Kühne has a carbol-methylene-blue made similar to the carbol-fuchsin.

Alkaline Stains.—Alkalis have the same object as the above agents, namely, to intensify the picture. Potassium hydroxid, ammonium carbonate, and sodium hydroxid are used.

Löffler’s alkaline blue and Koch’s weak alkaline blue have in them potassium.

Heat.—Warming or boiling the stains during the process of staining increases their intensity.

Decolorizing Agents.—The object after staining is usually overcolored in some part, and then *decolorizing* agents are employed. Water is sufficient in many cases; alcohol and strong mineral acids combined are necessary in some.

Iodin as Used in Gram’s Method.—Belonging to this group, but used more in the sense of a protective, is *tincture of iodine*. It picks out certain bacteria, which it coats; prevents *them* from being decolorized, but allows all else to be faded. Then, by using one of the acid or tissue dyes, a contrast color or double staining is obtained. Many of the more important bacteria are not acted upon by the iodine, and it thus becomes a very useful means of diagnosis.

FORMULAS OF DIFFERENT STAINING SOLUTIONS

I. *Saturated Alcoholic Solution.*

Place about 10 grams of the powdered dye in a bottle and add 40 grams of alcohol. Shake well and allow to settle. This can be used as the stock bottle.

II. *Weak Solutions.*

Made by adding about 1 part of stock solution (I) to 10 parts of distilled water. This is the ordinary solution in use.

III. *Anilin-oil Water.*

Anilin-oil	5 parts.	
Distilled water	100 "	—M.

Shake well and filter. To be made fresh each time.

IV. *Anilin-water Dyes.*

Saturated alcoholic solution of the dye	11 parts.	
Anilin-oil water	100 "	
Absolute alcohol	10 "	—M.

Can be kept ten days.

V. *Alkaline Methylene-blue.*A. *Löffler's:*

Saturated alcoholic solution methylene-blue	30.0	
Solution potassium hydroxid (1:10,000)	100.0.	—M.

B. *Koch's:*

Solution potassium hydroxid (10 per cent.)	0.2	
Saturated alcoholic solution methylene-blue	1.0	
Distilled water	200.0.	—M.

VI. *Phenol Solutions.*A. *Ziehl-Neelsen:*

Fuchsin (powdered)	1 part.
Alcohol	10 parts.
5 per cent. solution phenol	100 " —M.

Filter. The older the solution, the better.

B. *Kühne:*

Methylene-blue	1.5
Alcohol	10.0
5 per cent. solution phenol	100.0

Add the acid gradually. This solution loses strength with age.

VII. *Gram's Iodin Solution.*

Iodin	1.0
Potassium iodid.	2.0
Distilled water.....	300.0—M.

VIII. *Löffler's Mordant (for Flagella).*

Aqueous solution of tannin (20 per cent.)	10 parts.
Aqueous solution ferric sulphate (5 per cent.)	1 part.
Aqueous decoction of logwood (1 : 8) ..	4 parts.—M.

Keep in well-corked bottle.

IX. *Unna's Borax Methyl-blue.*

Borax.....	1 part.
Methyl blue	1 part.
Water	100 parts.—M.

X. *Gabbet's Acid Blue (Rapid Stain).*

Methylene-blue	2.0
25 per cent. sulphuric acid	100.0—M.

XI. *Alkaline Anilin-water Solutions.*

Sodium hydroxid (1 per cent.)	1.0
Anilin-oil water	100.0—M.
And add—	
Fuchsin, or methyl-violet powdered	4.0
Cork well. Filter before using.	

XII. *Roux's Double Stain.*

Dahlia or gentian-violet	0.5 gm.
Methyl-green	1.5 “
Distilled water	200.0 “ —M.
Use as other stains, without <i>acid</i> .	

XIII. *Neisser's Stain (for Diphtheria).*

Solution I.

Methylene-blue	1 gm.
Alcohol (96 per cent.)	20 c.c.
Dissolve and add—	
Water.	950 c.c.
Glacial acetic acid.	50 c.c.—M.

Solution II.

Vesuvium	2 gm.
Water	1000 c.c.—M.

Stain cover-glasses—(1) Three seconds in solution I; (2) wash in water; (3) three seconds in No. 2; (4) wash in water. Body of bacillus, brown; oval granules at each end, blue.

XIV. *Carbol-thionin (Nicolle).*

Saturated solution thionin in alcohol (90 per cent.)	10 c.c.
Aqueous solution phenol (1 per cent.)	100 c.c.—M.
Stain sections one-half to one minute.	

XV. *Capsule Stain of Hiss.*

Use the following, heated until it steams:

Saturated alcoholic solution of gentian-violet or fuchsin	5 c.c.
Distilled water	95 c.c.

Wash in 20 per cent. solution of cupric sulphate crystals.

XVI. *Capsule Stain of Welch.*

(1) Pour glacial acetic acid on film. After a few seconds replace with anilin-water gentian-violet without washing in water. (2) Remove all acid by several additions of stain, and allow it to act for three to four minutes. (3) Wash and examine in salt solution 0.8–2.0 per cent.

XVII. *Romanowsky Stains.*

A compound dye originally used for malarial parasites, but now employed in some of its modifications in staining blood-films, bacteria in tissues, and protozoa generally.

The stain is difficult to prepare, and can be purchased of supply houses to better advantage.

The chief modifications are:

Leishman's stain, consisting of a 1 per cent. solution methylene-blue, to which 0.5 per cent. sodium carbonate has been added and allowed to stand for twelve hours in incubator at 65° C., and then ten days at room temperature, and a solution of eosin (1 : 1000) in water. Equal parts of these solutions are mixed and allowed to stand for six hours. After it has been washed and dried, the precipitate is dissolved in methyl-alcohol.

Giemsa stain:

Azur II.—eosin	3 parts.
Azur II	8 “
Glycerin (pure)	250 “
Methyl-alcohol	250 “ —M.

Azur is a mixture of methylene-blue and eosin prepared in a special way.

Jenner's Stain.—See page 189.

J. H. Wright's stain: made in much the same way as Leishman's. The precipitate is not washed, but the saturated methyl-alcohol solution is filtered and further diluted with methyl-alcohol. The stains are used in very dilute form. Where the blood-films or exudates are not first fixed in alcohol, the concentrated stain is allowed to cover the preparation for five to twenty seconds to fix; then water is poured on to dilute and from five to fifteen minutes allowed for staining, the excess removed with water. The stains can be purchased in powder form, and need only be mixed with water to be ready for use.

CHAPTER V

GENERAL METHOD OF STAINING SPECIMENS

Cover-glass Preparations.—The material is evenly spread in as thin a layer as possible upon a cover-glass; then, to spread it still more finely, a second cover-glass is pressed down upon the first and the two slid apart. This also secures two specimens. Before they can be stained, they must be perfectly dry, otherwise deformities will arise in the structure.

Drying the Specimen.—The cover-glass can be set aside to dry, or held in the fingers over the Bunsen burner (the fingers preventing too great a degree of heat). Since most of the specimens contain a certain amount of albuminoid material, it is best in all cases to "fix"—*i. e.*, to coagulate the albumin. This is accomplished by passing the cover-glass (after the specimen is dry) three times through the flame of the burner, about three seconds being consumed in doing so, the glass being held in a small forceps, smeared side up.

The best forceps for grasping cover-glasses is a bent one, bent again upward, near the ends (Fig. 11). It prevents the flame or staining fluid from reaching the fingers.

The object is now ready for staining.

Staining.—A few drops of the staining solution are placed upon the cover-glass so that the whole specimen is covered, and is left on a few minutes, the time depending upon the variety, the strength of stain, and the object desired. Instead of placing the dye upon the object, the cover-glass can be immersed in a small glass dish containing the solution; or, if heat is desired to intensify or hasten the process, a watch-crystal holding the stain is placed over a Bunsen burner and in it the cover-glass; the cover-glass may be held directly in the flame with the staining fluid upon it, which must be constantly renewed until the process is completed, or the cover-glass can be heated in a test-tube, containing stain solution.

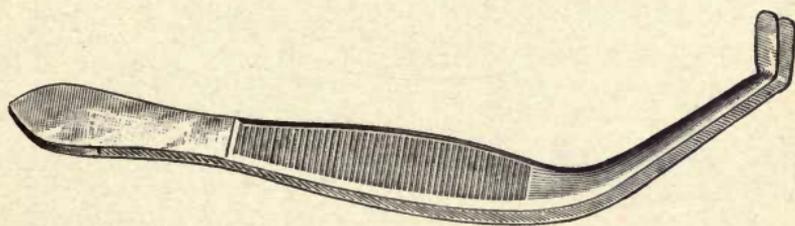


Fig. 11.—Author's bent forceps for holding cover-glass over flame.

Removing Excess of Stain.—The surplus stain is washed off by dipping the glass in distilled water.

The water is removed by drying between filter-paper or simply allowed to run off by standing the cover-glass slantwise against an object. When the specimen is to be examined in water (which is always best with the first preparation of the specimen, as the Canada balsam destroys to some extent the natural appearance of the bacteria), a small drop of sterilized water is placed upon the glass slide, and the cover-glass dropped gently down upon it, so that the cover-glass remains adherent to the slide.

The dry system or the oil immersion can now be used.

When the object has been sufficiently examined, it can be *permanently* mounted by lifting the cover-glass off the slide (this is facilitated by letting a little water flow under it, one end

being slightly elevated). The water that still adheres is dried off in the air or gently over the flame, and when perfectly dry, it is placed upon the drop of Canada balsam which has been put upon the glass slide.

In placing the cover-glass in the staining solutions one must be careful to remember which is the spread side, by holding it between oneself and the window and scraping the sides carefully with the sharp point of the forceps, the side having the specimen on it will show the marks of the instrument.

Little glass dishes, about one-half dozen, should be at hand for containing the various stains and decolorants.

Tissue Preparations.—In order to obtain suitable specimens for staining, very thin sections of the tissue must be made.

As with histologic preparations, the tissue must be hardened before it can be cut thin enough. Alcohol is the best agent for this purpose.

Pieces of the tissue one-quarter inch in size are covered with alcohol for twenty-four to forty-eight hours.

When hardened, it must be fixed upon or in some firm object. A paste composed of—

Gelatin	1 part.
Glycerin	4 parts.
Water	2 “

will make it adhere firmly to a cork in about two hours, or it can be embedded in a small block of paraffin, and covered over with melted paraffin. Celloidin may be used as an embedding agent, and formalin is useful to harden tissue quickly.

Cutting.—The microtome (Fig. 12) should be able to cut sections $\frac{1}{3000}$ inch in thickness; this is the fineness usually required.

The sections are brought into alcohol as soon as cut, unless they have been embedded in paraffin, when they are first washed in chloroform to dissolve out the paraffin.

Staining.—All the various solutions should be in readiness, best placed in the little dishes in the order in which they are to

be used, as a short delay in one of the steps may spoil the specimen.

A very useful instrument for transferring the delicate sections from one solution to another is a little metal spatula, the blade being flexible (Fig. 13).

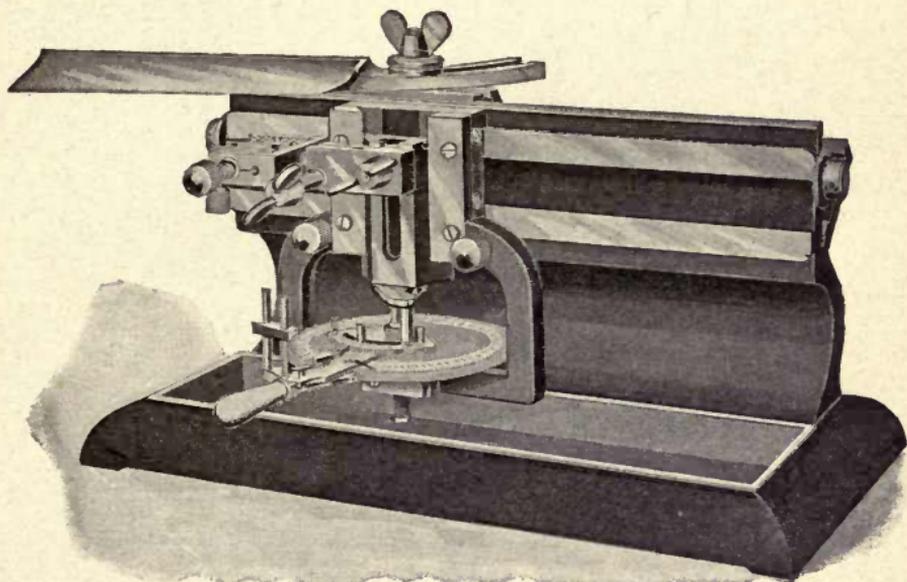


Fig. 12.—Large laboratory microtome (Mallory and Wright).

A still better plan, especially when the tissue is “crumbling,” is to carry out the whole procedure on the glass slide.



Fig. 13.—Spatula for lifting sections.

General Principles.—The section is transferred from the alcohol in which it has been kept into water, which removes the excess of alcohol, from here into—

Dish I, containing the *stain*, where it remains five to fifteen minutes. Then—

Dish II, containing 5 per cent. acetic acid (1 : 20), where it remains one-half to one minute. The acid removes the excess of stain.

Dish III, water, to rinse off the acid. The section can now be placed under the microscope, covered with cover-glass, to see if the intensity of the stain is sufficient or too great. A second section is then taken, avoiding the errors, if any; and having reached this stage, proceeded with as follows:

Dish IV, alcohol, two to three seconds, to remove the water in the tissue.

V. A few drops of *oil of cloves*, just long enough to clear the specimen to make it transparent (so that an object placed underneath will shine through).

VI. Remove excess with filter-paper.

VII. Mount in Canada balsam (xylol balsam).

CHAPTER VI

SPECIAL METHODS OF STAINING AND MODIFICATIONS

Gram's Method of Double Staining (*For Cover-glass Specimens*).—I. A hot solution of anilin-water gentian-violet two to ten minutes.

II. Directly, without washing, into Gram's solution of *iodin* potassium-iodid one to three minutes (the cover-glass looks black).

III. Wash in alcohol 60 per cent. until only a light brow shade remains (as if the glass were smeared with dried blood).

IV. Rinse off alcohol with water.

V. Contrast color with either eosin, picrocarmin, or Bismarck-brown. The bacteria will appear deep blue, all else red or brown on a very faint brown background.

Gram's Method for Tissues (*Modified by Günther*):

- I. Stain in anilin-water gentian-violet . . . 1 minute.
- II. Dry between filter-paper.
- III. Iodin potassium iodid solution 2 minutes.
- IV. Alcohol $\frac{1}{2}$ minute.
- V. 3 per cent. solution hydrochloric acid
in alcohol 10 seconds.
- VI. Alcohol, oil of cloves, and Canada balsam.

Behavior of the More Important Bacteria to Gram's Stain.—

Positive means that the bacteria retain the primary color, or gentian-violet, negative that they do not.

<i>Positive.</i>	<i>Negative.</i>
Tubercle bacillus.	Colon bacillus.
Smegma bacillus.	Typhoid bacillus.
Lepra bacillus.	Cholera bacillus.
Anthrax bacillus.	Influenza bacillus.
Tetanus bacillus.	Friedländer's bacillus.
Diphtheria bacillus.	Plague bacillus.
Pneumococcus.	Diplococcus intracellularis.
Streptococcus.	Gonococcus.
Staphylococcus.	Koch-Weeks' bacillus.
Cocci of the urethra.	Conjunctivitis bacillus of Morax.

To Stain Spores.—Since spores have a very firm capsule, which tends to keep out all external agents, a very intensive stain is required to penetrate them, but once this object is attained, it is equally as difficult to decolorize them.

A cover-glass prepared in the usual way, *i. e.*, drying and passing the specimen through the flame three times, is placed in a watch-crystal containing Ziehl's carbol-fuchsin solution, and the same placed upon a rack over a Bunsen burner, where it is kept at boiling-point for *one hour*, careful to supply fresh solution at short intervals lest it dry up.

The bacilli are now decolorized in alcohol containing $\frac{1}{2}$ per cent. hydrochloric acid. A contrast color, preferably methylene-blue, is added for a few minutes.

The spores will appear as little red beads in the blue-stained bacteria, and loose spores lying about outside the cell-wall.

Spore Stain (Modified).—I. *Carbol-fuchsin* on cover-glass and heated in the flame to boiling-point 20 to 30 times.

II. 25 per cent. sulphuric acid, two seconds; rinsed in water.

III. Methylene-blue contrast.

Alex. Klein recommends the following spore method: mix a little of the culture (potato) with three drops of physiologic salt solution, and heat gently with an equal quantity of carbol-fuchsin for a period of six minutes. Spread then on cover-glasses, dry in the air, and fix by passing three times through Bunsen-burner flame. Decolorize in 1 per cent. sulphuric acid for one to two seconds; contrast in weak methylene-blue.

Bowhill's Orcein Stain.

Saturated alcoholic solution of orcein . . . 15 c.c.

20 per cent. aqueous solution tannin . . . 10 c.c.

Distilled water 30 c.c.—M.

Filter.

Use orcein solution in watch-glass, float cover-glass in it, and heat gently, not boil, for ten minutes. Wash in water. Dry and mount in balsam.

Five per cent. chromium trioxid applied for fifteen minutes has been recommended in staining spores. This is followed by the carbol-fuchsin stain as above.

Sporogenic bodies stain quite readily, and in order to distinguish them from spores *Ernst* uses alkaline methylene-blue, slightly warmed. Then rinse in water. Contrast with cold Bismarck-brown.

The spores are colored bright blue, the spore granules a dirty blue, being mixed with the brown, which colors also the bacteria.

Kühne's Method.—In sections the alcohol used sometimes decolorizes too much. To obviate this *Kühne* mixes the alcohol with the stain, so that while the section is being anhydrated, it is constantly supplied with fresh dye.

Weigert uses anilin-oil to dehydrate instead of alcohol, and here, too, it can be used mixed with the dye.

Capsule Stain (*Buerger*).—I. Spread culture by means of a drop of ascitic fluid on cover-glass.

II. Fix in Müller's fluid, which has been saturated with 5 per cent. bichlorid of mercury, and warm for three seconds.

III. Wash quickly in water; rinse in alcohol.

IV. Cover with tincture of iodine for one minute.

V. Wash in alcohol and dry in air.

VI. Stain in anilin-water gentian-violet for two seconds.

VII. Wash in 2 per cent. salt solution.

VIII. Mount in salt solution ringed with vaselin.

Flagella Stain, with Löffler's Mordant.—I. A few drops of the mordant (No. VIII, p. 33) are placed upon the spread cover-glass and heated until it steams.

II. Wash with water until the cover-glass looks almost clean, using a small piece of filter-paper to rub off the crusts which have gathered around the edges.

III. Anilin-water fuchsin (neutral) held in flame about one and one-half minutes.

IV. Wash in water.

If the stain is properly made, the bacteria are deeply colored and the flagella seen as little dark lines attached to them.

Unna's Method for Fungi.—Especially useful for epidermic scales. Moisten horny scale or crust with acetic acid; macerate between two glass slides; dry in flame; wash out fat with ether and alcohol (equal parts); stain in *borax methyl blue* for ten seconds (over flame); bleach with glycerin and ether (equal parts); rinse in water, alcohol, dry and mount.

CHAPTER VII

METHODS OF CULTURE

Artificial Cultivation.—The objects of cultivation are to obtain germs in pure culture, free from all foreign matter, isolated, and so developed as to be readily used either for microscopic examination or animal experimentation.

To develop bacteria properly we supply, as nearly as possible, the conditions which hold for the especial germ in nature. With the aid of solid nutrient media the bacteria can be easily separated, and the methods are nearly perfect.

Sterilization of Cultures.—If we place our nutrient material in vessels that have not been properly disinfected, we will obtain growths of bacteria without having sown any.

If we have thoroughly cleaned our utensils and then not taken care to protect them from further exposure, the germs we have sown will be effaced or contaminated by multitudes of others that are constantly about us. We, therefore, have two necessary precautions to take:

First, thoroughly to clean and sterilize every object that enters into, or in any way comes in contact with, the culture.

Second, to maintain this degree of sterility throughout the whole course of the growth, and prevent, by proper containers, the entrance of foreign germs.

Disinfectants.—Corrosive sublimate (bichlorid of mercury), which is the most effective agent we possess, cannot be generally used because it renders the soil unproductive, and, therefore, must be employed only in washing dishes, to destroy the old cultures. Even after washing a few drops of the solution may remain and prevent growth, so that one must be careful to have the glassware that comes in contact with the nutrient media free from the sublimate.

Heat.—Heat is the best agent we possess for general use. Dry heat and moist heat are the two forms employed, but these differ greatly in effectiveness. Thus Koch found that while

moist heat at 100° C. killed the spores of the anthrax bacillus in one hour, it required three hours of dry heat at 140° C. to produce death.

For obtaining *dry heat*—that is, a temperature of 150° C. (about 300° F.)—a sheet-iron oven (Fig. 14) is used, which can be heated by a gas-burner. If it have double walls (air circulating between), the desired temperature is much more quickly obtained. A small opening in the top to admit a thermometer is necessary. These chests are usually about 1 foot high, $1\frac{1}{4}$ feet wide, and $\frac{3}{4}$ foot deep. In them glassware, cotton, and

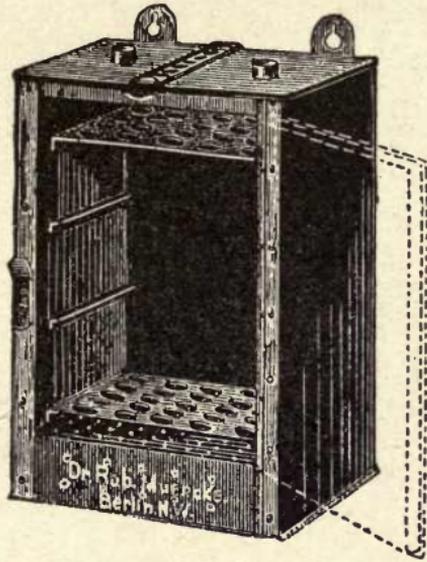


Fig. 14.—Hot air oven.

paper can be sterilized. When the cotton is turned slightly brown, it usually denotes sufficient sterilization. All instruments, where practicable, should be drawn through the flame of an alcohol lamp or Bunsen burner. One hour in the oven at 170° C. usually sterilizes glassware, while the ordinary germs in liquids may be killed by boiling for five minutes if no spores are present. The boiling of any fluid at 100° C. for one and one-half hours nearly always insures sterilization.

Moist Heat.—Steam at 100° C. in circulation has been shown to be a very effective application of heat.

Koch's Steam-chest (Fig. 15).—Circulating steam is obtained by aid of Koch's apparatus. This consists of a cylindrical tin chest about $2\frac{1}{2}$ feet high and about $\frac{1}{2}$ foot in diameter; divided in its interior by a perforated diaphragm, *a*, an upper cham-

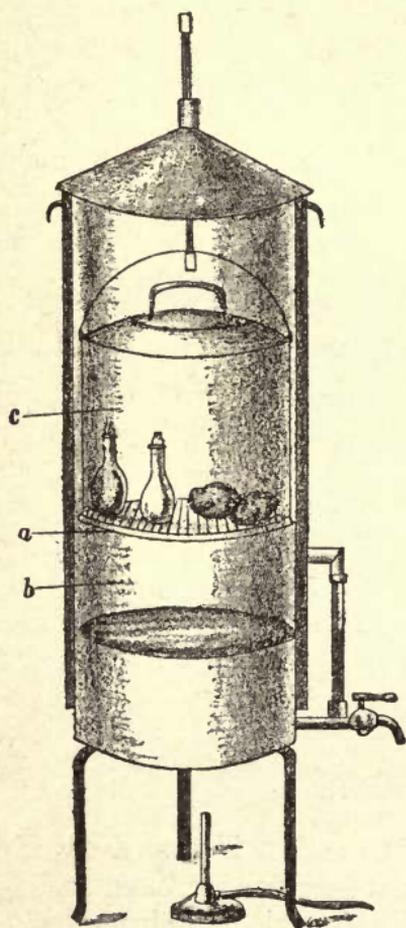


Fig. 15.—Koch's steam-chest.

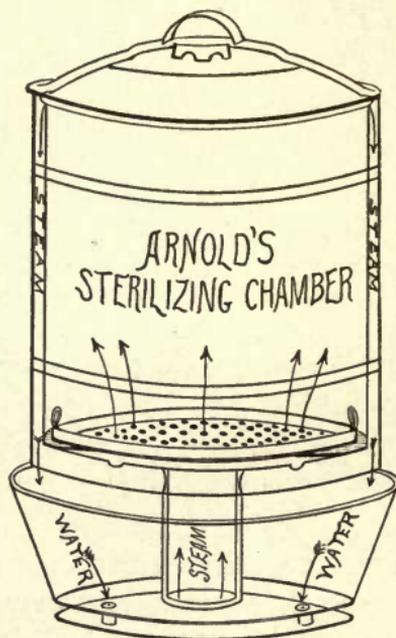


Fig. 16.—Arnold's steam sterilizer.

ber for the steam, *c*, and a lower one for water, *b*. Two or more gas-burners placed underneath the chest, which stands on a tripod, supply the heat. In the cover is an opening for a thermometer. The chest is usually covered with felt. When the thermometer registers 100°C ., the culture-medium or other sub-

stance to be sterilized is placed in the steam and kept there from ten to fifteen minutes, or longer, as required.

Arnold's steam sterilizer (Fig. 16) will answer every purpose of the Koch steam-chest. It is cheaper, also requiring less fuel to keep it going. The steam does not escape, but is condensed in the outer chamber.

The autoclave (Fig. 17), which produces steam under pressure and allows a temperature of 120° C. to be obtained, is a most effective method of sterilization, but the higher temperatures are not suitable for gelatin or sugar solution. Gelatin loses its power of solidifying if the boiling is prolonged.

Instead of sterilizing for a long time at once, successive sterilization is practised with nutrient media, so that the albumin will not be too strongly coagulated. Fifteen minutes each day for three days in succession in the steam-chest or autoclave is sufficient.

Fractional Sterilization of Tyndall.—Granted that so many spores originally exist in the object to be sterilized, it is subjected to 60° C. for four hours, in which time a part at least of those spores have developed into bacteria, and the bacteria destroyed by the further application of the heat. The next day more bacteria will have formed, and four hours' subjection to 60° C.

heat will destroy them, and so, at the end of a week, using four hours' application each day, all the spores originally present will have germinated and the bacteria be destroyed.

As modified, and in use in most laboratories, fifteen minutes'

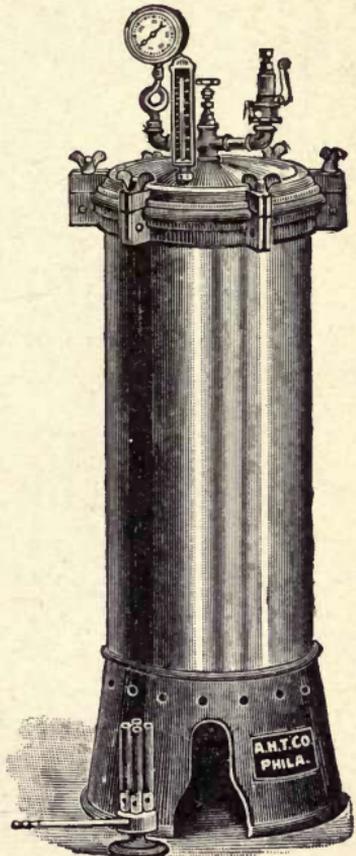


Fig. 17.—Modern autoclave.

sterilization in steam, at 100° C., on three successive days, has been found sufficient for nearly all purposes, while one sterilization in the autoclave at 110° C. for fifteen minutes will serve in some cases, especially if the media is for immediate use.

Cotton Plugs or Corks.—All the glass vessels (test-tubes, flasks, etc.) must be closed with cotton plugs, the cotton being easily sterilized and preventing the entrance of germs.

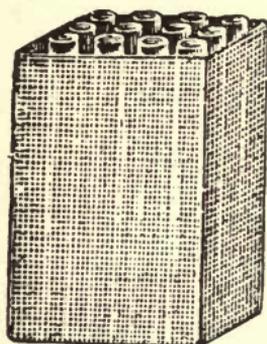


Fig. 18.—Wire cage.

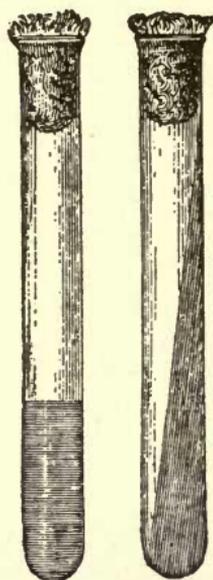


Fig. 19.—Cotton-plugged test-tubes.

Tin-foil may be used to cover the cotton, or caps made of india-rubber.

Test-tubes.—New test-tubes are washed with hydrochloric acid and water to neutralize the alkalinity often present in fresh glass. They are then well washed and rubbed with a brush, placed obliquely to drain, and when dry, corked with cotton plugs. Then put in the hot-air oven (little wire cages being used to contain them) for fifteen minutes, after which they are ready to be filled with the nutrient media. (The cotton should fit firmly in the tube and extend a short space beyond it.)

Test-tubes without flaring edges are more desirable, since the edges can easily be drawn out so as to seal the tube.

Instead of test-tubes, ordinary 3-ounce panel medicine bottles can be used for retaining the nutrient media and cultures.

According to late investigations, the glass tubes become sufficiently sterile in the steam-chest without the preliminary sterilization in the dry oven.

CHAPTER VIII

NUTRIENT MEDIA

OF the many different media recommended and used since bacteriology became a science, we can describe only the more important ones now in use. Each investigator changes them according to his taste.

FLUID MEDIA

Bouillon (According to Löffler).—A cooked infusion of beef made slightly alkaline with sodium carbonate: 500 grams of finely chopped raw lean beef is placed in a wide-mouthed jar and covered with 1 liter of water; this is left standing twelve hours with occasional shaking. It is then strained through cheese-cloth, the white meat remaining being pressed until one liter of the blood-red meat-water has been obtained. The meat-water must now be cooked, but before doing this, in order to prevent all the albumin from coagulating, 10 parts of peptone powder and 5 parts of common salt are added to every 1000 parts meat-water. For water analysis the salt must be omitted. It is next placed in the steam-chest or water-bath for three-quarters of an hour.

Neutralization.—The majority of bacteria grow best on a neutral or slightly alkaline soil, and the bouillon, as well as

other media, must be carefully neutralized with a saturated solution of sodium carbonate. Since too much alkalinity is nearly as bad as none at all, the soda must be added drop by drop until red litmus-paper commences to turn blue. The bouillon is then cooked another hour, and filtered when cold. The liquid thus obtained must be clearly alkaline, and not clouded by further cooking. If cloudiness occur, the white of an egg and further boiling will clear the same. To make bouillon, beef-extract can be used instead of fresh meat, 2 grams to 1 liter of water. This is boiled with 5 grams of salt and 10 of peptone, neutralized as above, and filtered when cold.

Schultz's Method of Neutralization.—A more accurate method of obtaining the required reaction is to use an alcoholic solution ($\frac{1}{3}$ per cent.) of phenolphthalein as an indicator; a few drops of this are mixed with 10 c.c. of the bouillon and 40 c.c. of water, and heated to boiling and while hot, $\frac{1}{20}$ normal sodium hydroxid from a buret is added, drop by drop, until a faint pink color appears. An average is taken from three different samples, and the amount of soda needed for the entire quantity of bouillon is calculated therefrom, and is added in the form of normal soda solution. The reaction is then expressed by the + sign if acid and the - sign if alkaline.

A bouillon that reacts neutral to litmus is, on an average, +25 to phenolphthalein, *i. e.*, requires 25 c.c. of normal soda solution to a liter to make it neutral to the latter indicator.

An optimum reaction is one about midway between the neutral point of litmus and the neutral point of phenolphthalein.

Glucose broth, which is a good medium for anaërobic organisms, consists of bouillon to which 1 to 2 per cent. of grape-sugar has been added. *Glycerin broth* is bouillon to which 6 to 8 per cent. of glycerin has been added after filtration.

Sterilization of the Bouillon.—Erlenmeyer flasks (little conic glass bottles) or test-tubes plugged and properly sterilized are filled one-third full with the bouillon, and placed with

their contents in the steam-chest. They are left in steam of 100° C. fifteen minutes for three successive days, after which the tubes and bouillon are ready for use.

Solid Media.—The knowledge of bacteria and germs or molds settling and growing upon slices of potato exposed to the air led to the use of solid media for the artificial culture of the same. It was thus learned that each germ tends to form a separate colony and remain isolated.

Potato-cultures.—A ripe potato with a smooth skin is the best.

Several are brushed and scrubbed with water to get rid of the dirt, and the “eyes” are cut out.

Next placed in 1 : 500 solution of bichlorid of mercury for one-half hour. Then in the steam-chest for three-quarters of an hour.

In the mean time a receptacle is prepared for them. This is called the *moist chamber*.

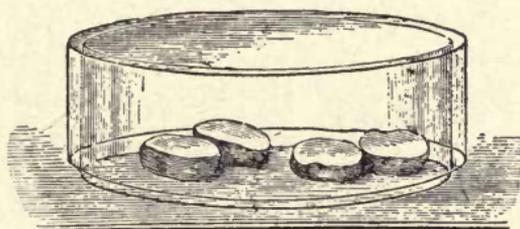


Fig. 20.—Moist chamber for potatoes.

The moist chamber consists of two large shallow dishes, one, the larger, as a cover to the other (Fig. 20).

These dishes are washed in warm distilled water.

A layer of filter-paper moistened with 15 to 30 drops of a 1 : 1000 bichlorid solution is placed in the bottom of the glass dish.

The operator now prepares his own hands, rolling up his coat-sleeves and carefully washing his hands, then taking a potato from the steam-oven and, holding it between his thumb and index-finger in the short axis, he divides the potato in its

long axis with a knife that has been passed through the flame. The two halves are kept in contact until they are lowered into the moist chamber, when they, of their own weight, fall aside, the cut surface uppermost. They are then ready for inoculation.

Esmarch's Cubes.—The potato is first well cleaned and peeled. It is then cut in cubes $\frac{1}{2}$ inch in size.

These are placed, each in a little glass dish or tray, and then in steam-chest for one-half hour, after which they are ready for inoculation (the dishes first having been sterilized in hot-air oven).

Test-tube Potatoes.—Cones are cut out of the peeled potato and placed in test-tubes, which can then be plugged and easily preserved.

Roux's test-tube (Fig. 21), specially designed for potato cultures, consists of a tube with a small constricted portion at the bottom, in which water may be kept to keep the potato moist.

Manner of Inoculation.—With a platinum rod or a spatula (sterilized) the material is spread upon one of the slices, keeping free of the edges. The growth on this first, or original, potato will be quite luxuriant, and the individual colonies often difficult to recognize; therefore dilutions are made (Fig. 22).

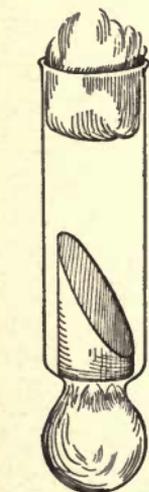


Fig. 21.—Tube for potato culture.

From the original or first slice a small portion, including some of the meat of the potato, is spread upon the surface of a second slice, which is first dilution. From this likewise a small bit is taken and spread on a third slice, or second dilution, and here usually the colonies

will be sparsely enough settled to study them in their individuality.

This is the principle carried on in all the cultivations. It is a physical analysis.

Potato and Bread Mash.—These pastes are used chiefly in the culture of molds and yeasts. Peeled potatoes are mashed

with distilled water until thick, and then sterilized in flasks three-quarters of an hour for three successive days.

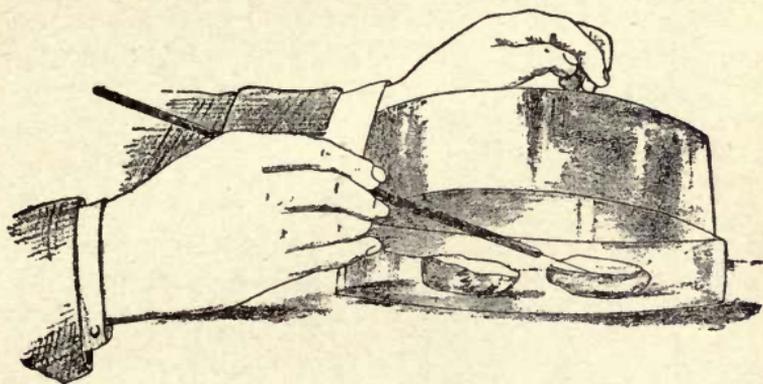


Fig. 22.—Method of inoculation (Woodhead and Hare).

Bread Mash.—Bread devoid of crust, dried in an oven, and then pulverized and mixed with water until thick, and sterilized as above.

CHAPTER IX

SOLID TRANSPARENT MEDIA

Solid transparent media are prepared from materials which can be used for microscopic purposes and which can readily be converted into liquids. Such are the gelatin and agar culture-media.

Gelatin.—Gelatin is obtained from bones and tendons, and consists chiefly of chondrin and gluten.

The French Golden Medal brand is the one most in use, found in long leaves with ribbed lines crossing them.

Koch-Löffler 10 per cent. Bouillon-gelatin.—To the meat-water as made for the bouillon are added 100 grams gelatin, 10 grams peptone, 5 grams salt, to each 1000 grams

of the meat-water; or to the bouillon made from beef-extract the gelatin is added; this is placed in a flask and gently heated until the gelatin is dissolved.

It is *neutralized* with the soda and then cooked in water-bath or carefully boiled over flame for one hour or more until the liquid seems clear, then add white of an egg and boil one-

quarter of an hour longer; the egg will produce a clearer solution and save much trouble. A small portion, while hot, is now filtered into a test-tube and tested for alkalinity, and then re-heated several times, watching if a cloudy precipitate forms.

If the fluid remains clear upon cooling, the remainder of the material can be filtered. It must be accomplished while hot, else the gelatin will coagulate and prevent further filtration.

This can be carried on either by keeping hot the solution continually in water-bath, and only filtering a small quantity at a time through the filter, or keeping the filter itself hot, either

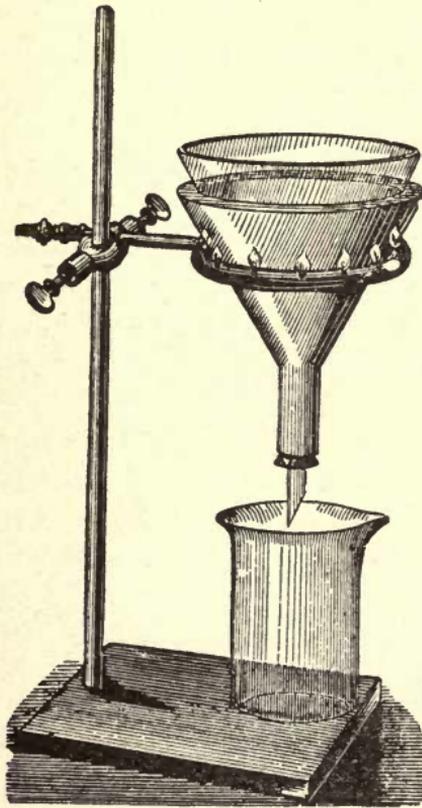


Fig. 23.—Hot-water filter.

with a hot-water filter or placing the filter in steam-chest (Fig. 23).

Clouding of Gelatin.—If the gelatin does not come out clear or becomes turbid on cooling, it may be due to several things—

1. The filter-paper too thin or impure.
2. Too strongly alkaline.
3. Cooked too long or not long enough.

The addition of the white of an egg, as before mentioned, will often clear it up; if this avails not, re-filtering several times and attention to the few points mentioned will produce a clear solution.

Sterilizing the Gelatin.—The gelatin is kept in little flasks or poured at once into sterile test-tubes, careful not to wet the neck where the cotton enters, lest when cool the cotton plug stick to the tube.

The tubes are then placed in steam-chest for three successive days, fifteen minutes each day (or in water-bath one hour a day for three days). Then set aside in a temperature of 15° to 20° C., and if no germs develop and the gelatin remains clear, it can be used for cultivation purposes.

Modification.—The amount of gelatin added to the meat-water can be variously altered, and instead of making gelatin bouillon, milk, blood, serum, urine, and agar can be added. Glycerin (4 to 6 per cent.) is a common addition, and sometimes reducing agents to absorb the oxygen are mixed with it.

Agar-agar.—This agent, which is of vegetable origin, derived from sea-plants gathered on the coasts of India and Japan, has many of the properties of gelatin, retaining its solidity at a much higher temperature; it becomes liquid at 90° C. and congeals again at 45° C. Gelatin will liquefy at 35° C.

Agar is not affected very much by the peptonizing action of the bacteria— 38° C. is the temperature at which most pathogenic germs grow best.

Preparation of Agar-agar Bouillon or Nutrient Agar.—The ordinary bouillon is first made, and then the agar cut in small pieces, added to the bouillon (15 grams of agar to 1000 grams of bouillon). It is allowed to stand several minutes until the agar swells, and then placed in water-bath or steam-chest for six hours or more. It is then neutralized, very little of the alkali being sufficient.

A white of an egg added, and boiled for several hours longer, when, even if not perfectly clear, it is filtered.

The filtering process, very difficult because of the readiness with which the agar solidifies, must be done in steam-chest or

with hot-water filter, and very small quantities passed through at a time, changing the filter-paper often.

Cotton can be used instead of filter-paper, or filtering entirely dispensed with, simply decanting.

As agar is seldom clear, a little more or less opaqueness is permissible. The test-tubes are filled as with the gelatin, and sterilized in the same manner. While cooling, some of the tubes can be placed in a slanting position, so as to obtain a larger surface to work upon.

Water of condensation will usually separate and settle at the bottom, or a little white sediment remain encysted in the center; this cannot easily be avoided, nor does it form any serious obstacle.

The crude agar should first be rinsed in water, and then in 5 per cent. acetic acid and clear water again, to rid it of impurities. If agar is boiled thoroughly over a hot flame or in an autoclave, it can be filtered much more readily. The main point is to see that all the agar is dissolved.

It has been suggested to pour the hot agar into high cylindrical glass vessels and allow it to cool slowly in the steam-oven, the flame having been gradually lowered and then turned out. After a time the cloudy portion will form a sediment at the bottom; the agar can then be shaken out as a long cylinder and the cloudy portion cut off.

The Japanese Method.—Yokote prepares agar as follows: the meat is cooked in water over a sand-bath one and one-half hours. Filtered, chopped agar is then added, and the mixture cooked one hour longer; peptone and salt added next. Neutralization. After the mixture has cooled to about 50° C., whites of two eggs are added and the mixture shaken thoroughly.

Again the mixture is placed on the sand-bath and heated to 110° C. and over for one and one-half to two hours, and then filtered through ordinary filter-paper. Yokote claims that by this procedure the agar can be filtered as easily as bouillon and without any loss. (Water must be added before filtering to supply loss from evaporation.)

The agar may be boiled separately in some of the water

ordinarily used in the meat-water, and then, when well dissolved, the concentrated meat-water infusion is added and the two solutions boiled together.

Glycerin-agar.—The addition of 4 to 6 per cent. of glycerin to nutrient agar greatly enhances its value as a culture-medium.

Gelatin-agar.—A mixture of 5 per cent. gelatin and 0.75 per cent. agar combines in it some of the virtues of both agents.

Blood-serum.—Blood-serum, being rich in albumin, coagulates very easily at 70° C., and if this temperature is not exceeded, a transparent, solid substance is obtained upon which the majority of bacteria develop, and some with preference.

Preparation of Nutrient Blood-serum.—If the slaughter of the animal can be supervised, it were best to have the site of the wound and the knife sterilized, and sterile flasks (Fig. 24) at hand to receive the blood directly as it flows.

The blood is placed on ice forty-eight hours, and the serum is drawn out with sterile pipets into test-tubes; these are placed obliquely in an oven where the temperature can be controlled and maintained at a certain degree. (See Fig. 25.)

Incubators or Brood-ovens.—Incubators or brood-ovens consist essentially of a double-walled zinc or copper chest, the space between the walls being filled with water.

The oven is covered with some impermeable material to prevent the action of the surrounding atmosphere (Fig. 26). It is supplied with a thermometer and a regulator. The regulator is connected with a Bunsen burner, and keeps the temperature at a certain height.

There are several forms of regulators in use, and new ones are invented continually. The size of the flame in some is regulated by the expansion of mercury, which, as it rises, lessens the opening of the gas-supply. The mercury contracting on cooling allows more gas to enter again (Fig. 27).

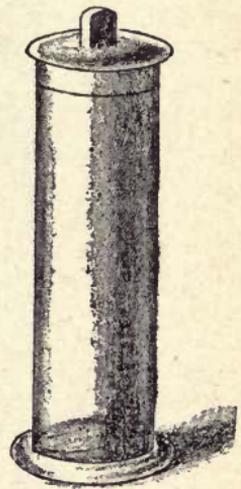


Fig. 24.—Flask to receive blood-serum.

Koch has invented a *safety burner* by which the gas supply is shut off should the flame accidentally go out.

Coagulation of Blood-serum.—The tubes of blood-serum having been placed in the oven, are kept at a temperature of 65° to 68° C. until coagulation occurs; then removed and sterilized.

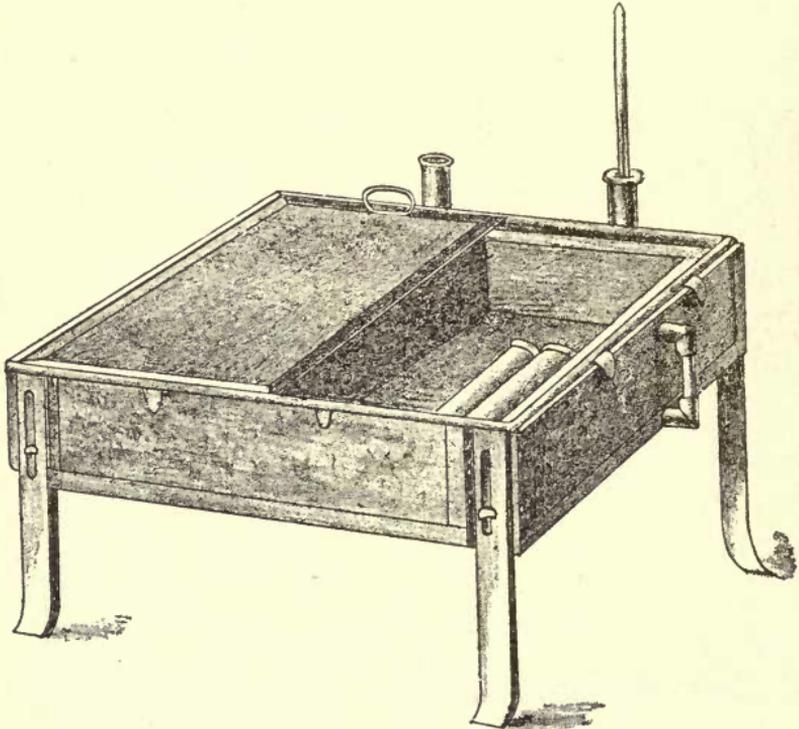


Fig. 25.—Thermostat for blood-serum.

Sterilization of Blood-serum.—The tubes are placed three to four days in incubation at 58° C., and those tubes which show any evidences of organic growth are discarded.

If, now, at the end of a week, the serum remains sterile at the ordinary temperature of the room, it can be used for experimental purposes.

Perfectly prepared blood-serum is transparent, of a gelatin-like consistence, and straw color. It will not liquefy by heat, though bacteria can digest it. Water of condensation always forms, which prevents the drying of the serum. Blood-serum

may be prepared in a shorter way by coagulating the serum at a temperature short of boiling-point. Sterilization is completed in three days by exposing the tubes to a temperature of about 90° C. each day for five minutes. Tubes so prepared are opaque and white.

Preservation of Blood-serum in Liquid State.—Kirchner advises the use of chloroform. To a quantity of serum in a

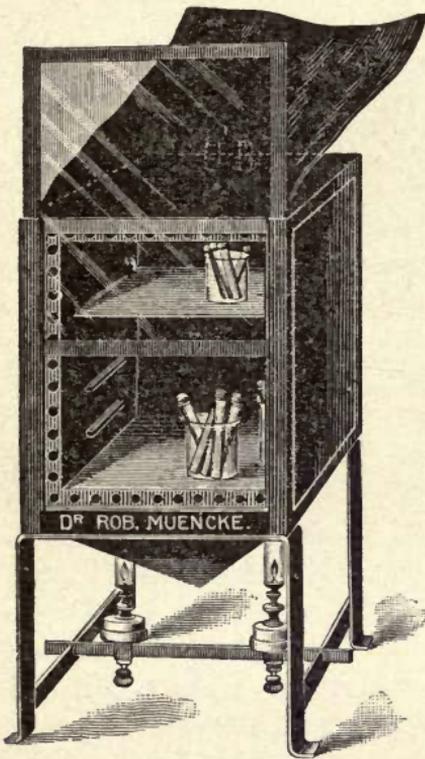


Fig. 26.—Incubator.

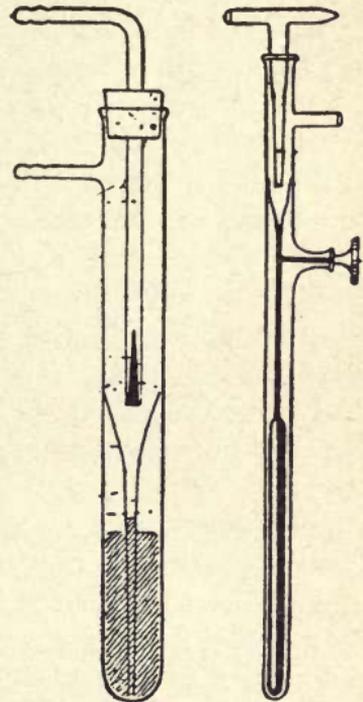


Fig. 27.—Thermoregulators.

well-stoppered flask a small amount of chloroform is added—enough to form about a 2 mm. layer on the bottom. If the chloroform is not allowed to evaporate, the serum remains sterile for a long time. When needed for use, test-tubes are filled and placed in a water-bath at 50° C. until all chloroform has been driven off (determined by absence of characteristic odor); the serum is then solidified and sterilized as in the ordinary way.

Human blood-serum derived from placenta, serum from ascitic fluid and ovarian cysts, is prepared in a similar manner to the above.

Blood coagulum, suggested by the author, is the blood itself (not the serum only) coagulated in test-tubes. It is dark brown in color and allows some colonies of bacteria to be more visible. It requires less time to prepare, and is not so likely to become contaminated as when the serum is used.

Löffler's Blood-serum Mixture.—See p. 124.

Peptone Solution (Dunham's).—Sodium chlorid, 0.5 parts; peptone, 1 part; water, 100 parts. Boil, filter, and sterilize. Useful to detect presence of indol.

Other Nutrient Media.—Milk, urine, decoctions of various fruits and plants, and for cultivating anaërobic bacteria, eggs.

Lactose (or Dextrose) Litmus-agar (for Water Bacteria).—To nutrient agar, 1 per cent. of dextrose or lactose is added just before sterilization. The reaction should be *neutral* to phenolphthalein. Then, if the medium is to be used in tubes, sterilized azolitmin, 1 per cent. (aqueous solution) is added just before the final sterilization. If Petri dishes are used, the azolitmin solution is not added until the medium is ready to be poured.

Blood-agar.—Human or other blood is obtained direct from the body under strict aseptic conditions, and a few drops smeared over the surface of agar in tubes or plates. These are then placed in the incubator for a few days, and the contaminated ones are rejected. This media is used for influenza bacilli and gonococci.

Dunham's rosalic acid solution consists of the following:

Peptone solution (Dunham).....	100 c.c.
2 per cent. solution rosalic acid.....	0.5 gm.
Alcohol (80 per cent.).....	100 c.c.—M.

To detect acids and alkalis.

Elsner's Medium (for Typhoid) (Potassium Iodid—Potato-gelatin).—Five hundred grams of peeled and washed

potatoes are mashed and pressed through a fine cloth. The juice is allowed to settle, is filtered, and after one hour's cooking has added to it 10 per cent. gelatin; then $2\frac{1}{2}$ c.c. $\frac{1}{10}$ normal sodium hydroxid solution, and finally 1 per cent. potassium iodid.

Typhoid Medium of Hiss.—This consists of a slightly acid mixture of gelatin and agar, beef-extract, sodium chlorid, and dextrose, used in different proportions for plate and tube cultures. It is semisolid in character, and facilitates the identification of the motile typhoid bacilli, which produce a uniform clouding through the medium in tubes.

Bile-salt Media (MacConkey).—Used for intestinal bacteria; stock solution consists of:

Sodium taurocholate	0.5 gm.
Witte's peptone.....	2.0 “
Distilled water.....	100.0 c.c.—M.

To which is added as an indicator, neutral red (crimson with acid, yellow with an alkali) in 1 per cent. solution, 0.5 c.c., and dextrose or lactose. The fluid is placed in fermentation tubes and sterilized for ten minutes on three successive days.

Agar can be added to make bile-salt lactose agar. *Bacillus coli* and *Bacillus typhosus* grow readily on this media; other water bacteria are inhibited, especially at 40° C.; acid-formers and gas-formers are denoted by rose-red-colored colonies.

Milk Culture-medium.—The milk used should be fresh and should be placed on ice for eight to ten hours to allow the cream to rise; the skimmed milk is siphoned off into flasks or tubes and sterilized for three successive days. *Litmus* is often added, or sterile 1 per cent. *azolitim* solution.

Urine Media (for Gonococci):

Urine (sterile taken).....	1 part.
2 per cent. agar solution.....	1 “

Fresh Egg Cultures (After Hueppe).—The eggs in the shell are carefully cleaned, washed with sublimate, and dried with cotton.

The inoculation occurs through a very fine opening made in the shell with a hot platinum needle; after inoculation, the opening is covered with a piece of sterilized paper, and collodion.

Boiled Eggs.—Eggs boiled, shell removed over small portion, and the coagulated albumen stroked with the material.

Guinea-pig Bouillon.—The flesh of guinea-pigs, as well as that of other experiment animals, is used instead of beef in the preparation of bouillon, for the growth of special germs.

The extracts of different organs have been added to the various media for experimentation.

Fermentation Tube.—For showing the presence of gas or fermentation the Smith tube (Fig. 28) or some of its modifications must be used. The closed end and part of the bulb are filled with the glucose or dextrose bouillon and

sterilized at low temperatures for three successive days, then inoculated, and placed in the incubator. Gas forms gradually, displacing the fluid in the closed end.

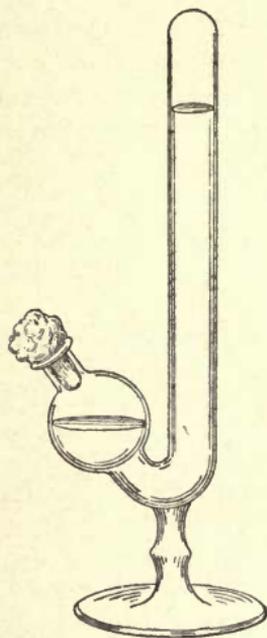


Fig. 28.—Smith's fermentation tube.

CHAPTER X

INOCULATION OF GELATIN AND AGAR

Glass Slide Cultures.—Formerly the gelatin was poured on little glass slides, such as are used for microscopic purposes, and after it had become hard, inoculated in separate spots as with potatoes.

Test-tube Cultures.—The gelatin, agar, or blood-serum having solidified in an oblique position, is smeared on the surface with the material, and the growth occurs, or the medium is punctured with a stab of the platinum rod containing the material. The former is called a *stroke* or *smear culture*, the latter

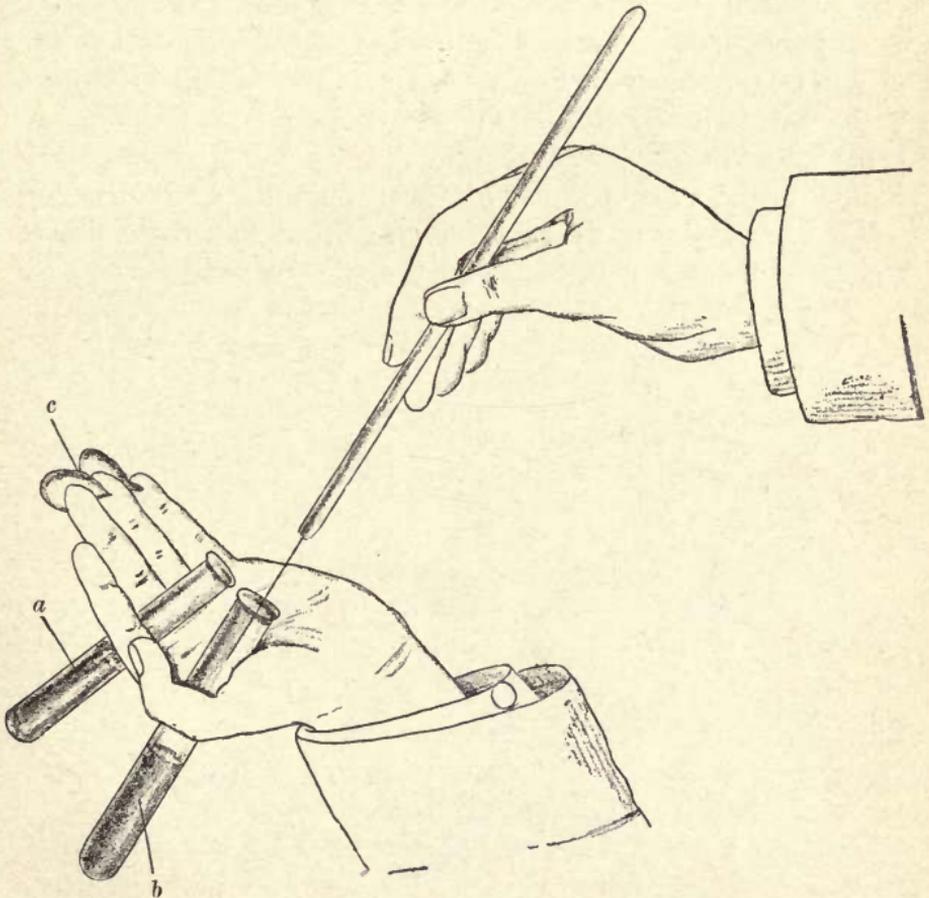


Fig. 29.—Manner of holding tubes for inoculation: *a*, Tube with material; *b*, tube to be inoculated; *c*, cotton plugs (after Woodhead and Hare).

a *stab* or *thrust culture*. In removing the cotton plugs from the sterile tubes to carry out the inoculation, the plugs should remain between the fingers in such a way that the part which comes in contact with the mouth of the tube will not touch anything (Fig. 30).

It is well to pass the mouth of the tube and the cotton plugs through a flame, scorching the latter before reinserting.

After the needle has been withdrawn, the plugs are reinserted and the tubes labeled with the kind and date of culture.

Plate Cultures.—This method once common is now seldom or ever used, and has been superseded by the Petri dishes; as a matter of history the description is retained. Several tubes of the culture-medium are made liquid by heating in water-bath, and then inoculated with the material as follows. A looped platinum needle is dipped into the material and then shaken in the tube of liquid media (gelatin, agar, etc.).

This first tube is called *original*. From this three drops (taken with the looped platinum rod) are placed in a second

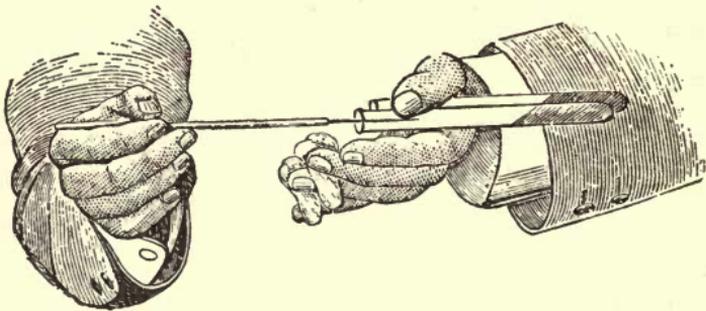


Fig. 30.—Manner of holding plugs.

tube, the rod being shaken somewhat in the gelatin or agar; this is labeled *first dilution* (a colored pencil is useful for such markings). From the first dilution three drops are taken into a third tube, which becomes the *second dilution* (Fig. 29).

The plugs of cotton must be replaced after each inoculation, and during the same must be carefully protected from contamination.

Glass Plates.—The larger the surface over which the nutrient medium is spread, the more isolated will the colonies be; window glass cut in rectangular plates 6 x 4 inches in size is used; about ten such plates are cleaned with dry towel and placed in a small iron box or wrapped in paper, and steril-

ized in the hot-air oven at a temperature of 150° C. for ten minutes (Fig. 31). When the plates have cooled, they are placed upon an apparatus designed to cool and solidify the liquid media, which is now poured upon the plates from the inoculated test-tubes.

Nivellier Leveling and Cooling Apparatus.—Ice and water are placed in a shallow round glass tray; on top of this a square plate of glass, upon which the culture-plate is placed, and covering this, a bell-glass.

The whole is upon a low wooden tripod, the feet of which can be raised or lowered, and a little spirit-level used to adjust it (Fig. 32). The glass plate taken out of the iron box is placed under the bell-glass. The tube containing the gelatin is held in the flame a second to singe the cotton plug to free it from dust, and the plug removed, the edges of the tube again flamed, the bell-glass

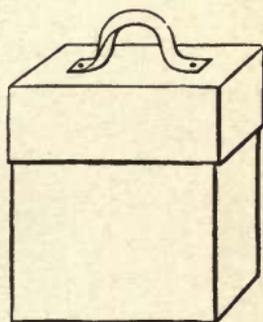


Fig. 31.—Iron box for glass plates.

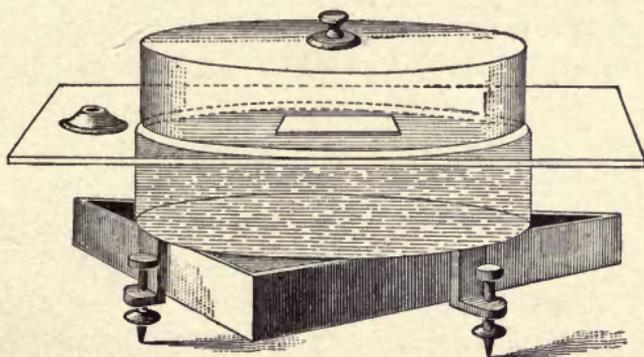


Fig. 32.—Nivellier leveling and cooling apparatus.

lifted, and the inoculated gelatin carefully poured on the plate, leaving about one-third inch margin from the borders; the lips of the tube being sterile, can be used to spread the media evenly. If the plate is at all cool, the fluid will solidfy as it is being spread. The glass cover is replaced until the gelatin or agar is quite solid, to prevent contamination.

When the gelatin is congealed, the plate is placed upon a little glass bench or stand in the moist chamber.

The Moist Chamber.—Prepared out of two glass dishes, as for the potato-cultures. The glass benches are so arranged that one stands upon the other (Fig. 33). In order to avoid confusion, a slip of paper with a number written on it is placed

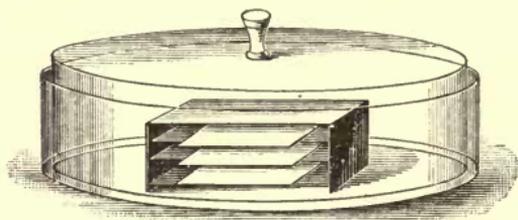


Fig. 33.—Moist chamber with plates on benches.

on the bench beneath each plate. As the original or first plate would have the colonies developed in greatest profusion, it is placed the first day on the topmost bench; but, since the colonies would be likely to overrun the plate and allow the gelatin to drop on the lower plates, it is best, as soon as evidences of growth appear, to place it below, and watch the third plate or second

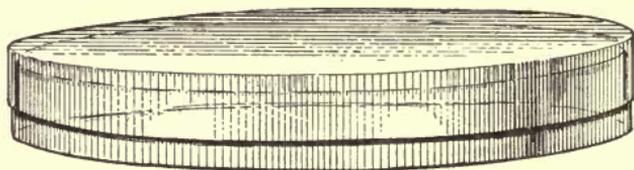


Fig. 34.—Petri dish for making plate cultures.

dilution for the characteristic colonies, forgetting not all this time to change the numbers accordingly.

The date of culture and the name can be written upon the moist chamber.

Petri Saucers.—Agar hardens very quickly, even without any especial means for cooling, and it does not adhere very well to the glass. Therefore it is better to follow the method

of Petri and use little shallow glass dishes, one covering the other (Fig. 34). They are first sterilized by dry heat, and then the inoculated gelatin or agar is poured into the lower dish, covered by the larger one, and placed in some cool place, different saucers being used for each dilution.

This method is very useful for transportation, and does away with the cooling apparatus and moist chamber; the saucers can be viewed under microscope similar to the glass plates, and have entirely superseded them.

Esmarch's Tubes or Rolled Cultures.—This method, especially used in the culture of anaërobic germs, consists in spreading the inoculated gelatin upon the inner walls of the test-tube in which it is contained and allowing it to congeal. The colonies then develop upon the sides of the tube without the aid of other apparatus. The method is useful whenever a very quick and easy way is required. The rolling of the tube is done under ice-water or running water from the faucet. The tube is held a little slanting, so as to avoid getting too much gelatin around the cotton plug.

The tubes can be placed directly under the microscope for further examination of the colonies.

Animals as Culture Media.—It is almost impossible to separate certain organisms, such as the tubercle bacillus and pneumococcus, from mixed cultures by ordinary plate methods, and the plan of producing the disease in animals by inoculation, and then obtaining the organism in pure culture, has to be employed.

Pure Cultures by Boiling.—Spored organisms may be separated from others by boiling the mixture for a few minutes, when all the non-spored forms will perish, and only the spores remain to germinate subsequently.

CHAPTER XI

THE GROWTH AND APPEARANCES OF COLONIES

Macroscopic.—Depending greatly upon the temperature which should be about 65° F. (20° C.) for gelatin, and 40° C. for agar, the colonies ordinarily develop so as to be visible to the naked eye in two to four days. Some require ten to fourteen days, and others grow rapidly, covering the third dilution in thirty-six hours. The plate should be looked at each day.

The colonies present various appearances from that of a small dot, like a fly-speck, to that resembling a small leaf.

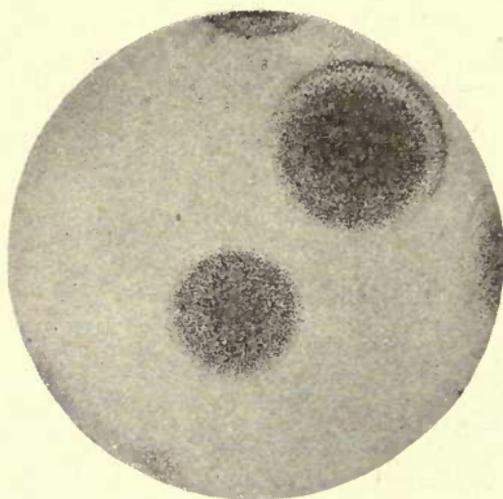


Fig. 35.—*Staphylococcus pyogenes aureus*: colony two days old, seen upon an agar-agar plate ($\times 40$) (Heim).

Some are elevated, some depressed, and some, like cholera, cup-shaped—umbilicated.

Then they are variously pigmented. Some liquefy the gelatin speedily, others not at all. The appearances of a few are so characteristic as to be recognized at a glance.

Microscopic.—We use a low-power lens, with the Abbé nearly shut out—that is the narrowest blender. The stage of

the microscope should be of such size as to carry a culture plate easily upon it.

The second dilution or third plate is usually made use of—that one containing the colonies sufficiently isolated.

These isolated ones should be sought for, and their appearance well noticed.

There may be two or three forms from the same germ, the difference due to the greater or less amount of oxygen that they have received, or the greater or less amount of space that they have had to develop in.

The microscopic picture varies greatly; now it is like the gnarled roots of a tree, and now like bits of frosted glass; the

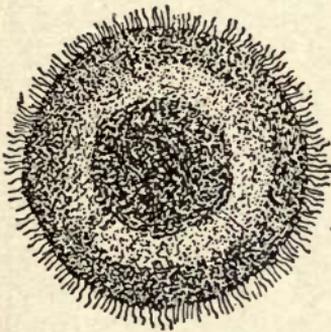


Fig. 36.—Microscopic appearances of colonies

Fig. 37.—Klatsch preparations.

pictures are very characteristic, and the majority of bacteria can be told thereby (Fig. 35).

Impression or "Klatsch" Preparations.—In order more thoroughly to study a certain colony and to make a permanent specimen of the same, we press a clean cover-glass upon the particular colony, and it adheres to the glass. It can then be stained or examined so. The Germans give the name of "Klatsch" to such preparations. Many beautiful pictures can be so obtained.

Fishing.—To obtain and examine the individual members of a particular colony the process of fishing, as it is called, is resorted to.

The colony having been placed under the field of the microscope, a long platinum needle, the point slightly bent, is passed between the lens and the plate so as to be visible through the microscope, then turned downward until the colony is seen to

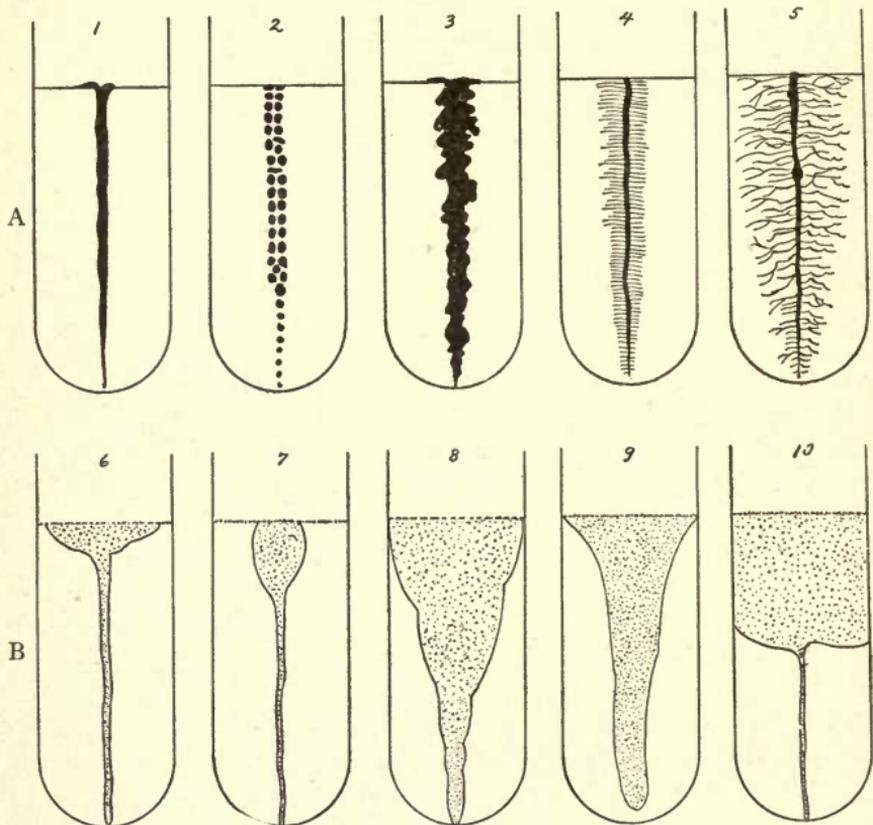


Fig. 38.—Types of growth in stab-cultures: A, Non-liquefying: 1, Filiform (*Bacillus coli*); 2, beaded (*Streptococcus pyogenes*); 3, echinate (*Bacterium acidi lactici*); 4, villous (*Bacterium murisepticum*); 5, arborescent (*Bacillus mycoides*). B, Liquefying: 6, Crateriform (*Bacillus vulgare*, twenty-four hours); 7, napiform (*Bacillus subtilis*, forty-eight hours); 8, infundibuliform (*Bacillus prodigiosus*); 9, saccate (*Microsporion Finkleri*); 10, stratiform (*Psorospermum fluorescens*) (Frost).

be disturbed, and the needle is dipped into the colony. This procedure must be carefully done, lest a different colony be disturbed than the one looked at, and an unknown or unwanted germ obtained.

After the needle has entered the particular colony, it is with-

drawn, and the material thus obtained is further examined by staining and animal experimentation. The bacteria are then again cultivated by inoculating fresh gelatin, making *stab*- and *stroke* cultures.

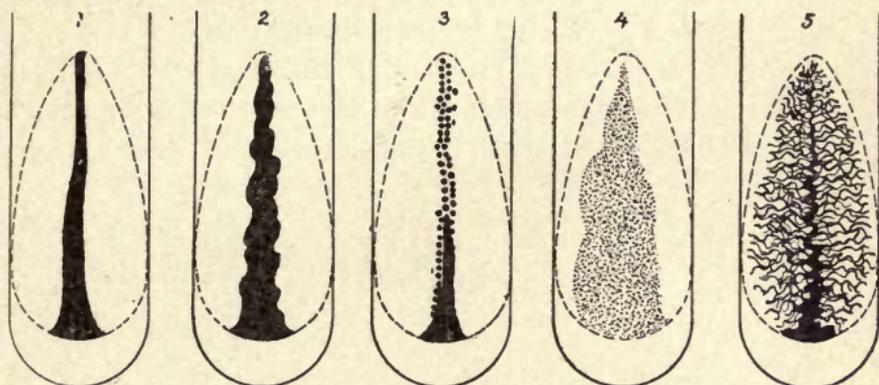


Fig. 39.—Types of streak cultures: 1, Filiform (*Bacillus coli*); 2, echinulate (*Bacterium acidi lactici*); 3, beaded (*Streptococcus pyogenes*); 4, effuse (*Bacillus vulgaris*); 5, arborescent (*Bacillus mycoides*) (Frost).

It is necessary to transfer the bacteria to fresh media about every six weeks, as the products of growth and decay given off by the organisms destroy them. Stroke and stab test-tube cultures are more characteristic than plate cultures, as the types in figures 38 and 39 show.

CHAPTER XII

CULTIVATION OF ANAËROBIC BACTERIA

SPECIAL methods are necessary for the culture of the anaërobic variety of bacteria in order to procure a space devoid of oxygen.

Liborius's High Cultures.—The tube is filled about three-quarters full with gelatin, which is then steamed in a water-bath and allowed to cool to 40° C., when it is inoculated by

means of a long platinum rod with small loop, the movement being a rotary vertical one, and the rod going to the bottom of the tube.

The gelatin is next quickly solidified under ice; very little air is present. The anaërobic germs will grow from the bottom upward, and any aërobins present will develop first on top, this method being one of isolation.

From the anaërobic germ grown in the lower part, a stab-culture is made into another tube containing three-quarters

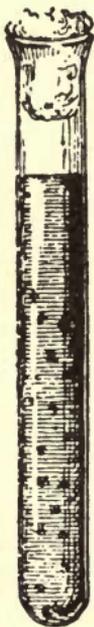


Fig. 40.—Liborius's method.



Fig. 41.—Hesse's method of making anaërobic cultures (McFarland).

gelatin, the material being obtained by breaking test-tube with the culture. (See Fig. 40.)

Hesse's Method.—A stab-culture having been made with anaërobic germs, gelatin in a semisolid condition is poured into the tube until it is full, thus displacing the air (Fig. 41).

Esmarch's Method.—Having inoculated a tube, the gelatin is rolled out on the walls of the tube, a "roll culture," and the rest of the interior is filled with gelatin, the tube

being held in ice-water. The colonies develop upon the sides of the tube and can be examined microscopically.

Gases like Hydrogen to Replace the Oxygen.—Several arrangements for passing a stream of hydrogen through the culture:

Fränkel puts in the test-tube a rubber cork containing two glass tubes, one reaching to the bottom and connected with a hydrogen apparatus, the other very short, both bent at right angles. When the hydrogen has passed through ten to thirty minutes, the short tube is annealed and then the one in connection with the hydrogen bottle, and the gelatin rolled out upon the walls of the tube (Fig. 42).

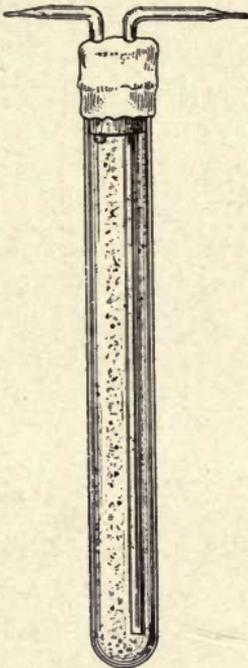


Fig. 42.—Fränkel's method of making anaërobic cultures (McFarland).



Fig. 43.—Buchner's method of making anaërobic cultures (McFarland).

Use of Aërobic Bacteria to Remove the Oxygen.—Roux inoculates an agar tube through a needle-thrust, after which semisolid gelatin is poured in on top. When the gelatin has

solidified, the surface is inoculated with a small quantity of *Bacillus subtilis* or some other aërobic germ. The *subtilis* does not allow the oxygen to pass by, appropriating it to itself.

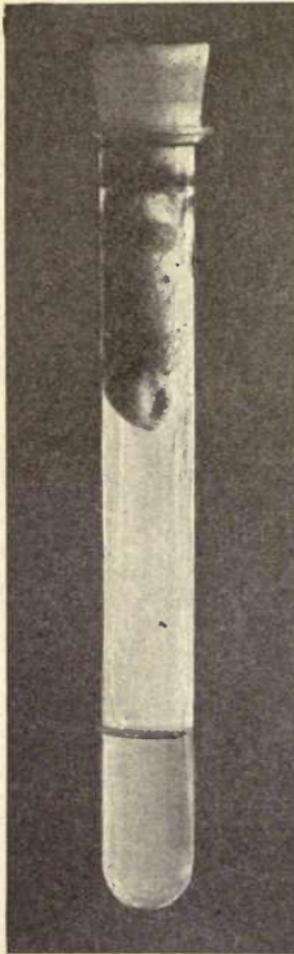


Fig. 44.—Wright's method for the cultivation of anaërobes.

Buchner's Method.—The test-tube containing the culture is placed within a larger tube, the lower part of which contains an alkaline solution of pyrogallic acid. The tube is then closed with a rubber stopper (Fig. 43).

Botkin's Method.—Petri dishes, uncovered, are placed on a rack under a large bell-jar, into which hydrogen gas is conducted. Alkaline pyrogallic acid is placed in the upper and lower dishes to absorb what oxygen remains.

Wright's Method.—Applicable to both fluid and solid media. After inoculating the test-tube, the plug, which must be of absorbent cotton, is cut off flush with the extremity of the tube, and pushed inward for a distance of 1 cm. It is then impregnated with 1 c.c. of a watery solution of pyrogallic acid and 1 c.c. of 5 per cent. sodium hydroxid solution. A tightly fitting rubber stopper is inserted, and the tube is then ready for incubation. (See Fig. 44.)

Park's Method.—An Erlenmeyer flask containing the medium to be used is boiled in a water-bath from ten to fifteen minutes to drive off dissolved oxygen, quickly cooled, and inoculated.

Hot melted paraffin is then poured into the flask, which forms a layer over the medium, and on congealing, provides an air-tight seal which does not adhere to the glass so closely as to prevent the escape of any gases formed by the bacterial growth.

CHAPTER XIII

INFECTION

How Bacteria Cause Disease.—Many theories have been advanced to explain the action of bacteria in causing disease, but only a few of the more important ones can be taken up here. Nearly all the changes found in the organs of the body are similar to those produced by drugs and can be reproduced by the injection of bacterial poisons.

What are the Conditions Necessary to Produce Infection?

First, as to the infective agent. The organism must have the power to produce disease. It must, in other words, be *pathogenic*. A non-pathogenic bacterium under certain conditions may cause disease, but this is not an infectious disease; it is due to the absorption of poisons generated outside of the body. It must be *parasitic*—have the power of growing within the body of an animal.

Essentially an infectious disease is a toxemia, because it depends upon poisons or toxins produced in the body. *Parasitic* or *infectious bacteria* cause disease by growing in the animal organism and generating products therein which are toxic. *Saprophytic bacteria* grow *outside* of the animal organism in dead matter, decaying particles, etc., and they may give rise to products which also are toxic to the animal economy.

Second, the toxins or poisons elaborated must be present in sufficient amount. Undoubtedly each animal organism is a law unto itself in regard to the amount of poison it will tolerate before disease is actually produced. The period of incubation can be explained on the supposition that the germ requires so much time to elaborate the amount of toxin necessary. This time period varies with different organisms, some carrying the toxin with them at the time of entry.

Third, the animal infected must be susceptible. Susceptibility varies in different species of animals, in different members of the same species, in the same individual at different times, and

in the same individual to the different forms of disease germs. *Susceptibility* may be *natural* to the race, it may be *acquired*, it may be *inherited*. Mice are naturally susceptible to anthrax. *Acquired* susceptibility occurs upon exposure to conditions which lower vitality, as hunger, cold, advanced age, and surgical shock. *Inherited susceptibility* is a less important factor now than formerly. Many diseases were at one time considered inherited which now are known to be acquired during the lifetime of an individual. Still, certain physical characteristics, such as narrow chest, mouth-breathing, etc.—clearly inheritable characters—predispose to disease. Given a susceptible individual and an infective microorganism producing toxins in sufficient amount, disease is certain to result.

Fourth, the infecting organism must gain entrance into the tissues or find lodgment on some portion of the body that has been injured; on the skin, in the nose, and in the throat are at all times bacteria that are more or less pathogenic, but they fail to cause disease until, through a traumatism or lessened resistance, they are allowed to enter the tissues or the blood and then they act.

Local Effects of Bacteria.—By *mechanical obstruction* from rapid growth, thrombosis, with its consequences, may occur. *Destruction of a part* of the cells of a tissue with necrosis can arise from irritation, as from a foreign body.

General Effects.—*Sapremia*, when toxic products of local suppuration are absorbed into the system. *Septicemia*, when the infective agent itself enters the blood-stream and causes general disturbance.

Suppurative bacteria are those which give rise to inflammation and suppuration locally at the point of entrance, and secondarily through metastasis. Any organism may cause suppuration, but a number are peculiarly inclined to give rise to pus, and are known as *pyogenic* organisms.

Specific Bacteria.—*Infective bacteria* are, as a rule, *specific*, the particular toxin having a specific action and causing a disease peculiar to the microorganism. Thus typhoid fever is a disease distinctly different from tuberculosis; the infective

organisms are distinct and the poisons they produce have specific characteristics.

The Nature of Toxins.—Very similar to the venom of serpents; highly poisonous in minute doses ($\frac{1}{10000}$ gram of tetanus toxin will kill a horse weighing 600 kilos—1200 pounds). At first toxins were called *ptomains*, or cadaveric alkaloids; but this term is applied now to such poisons as have a *basic* nature and arise in decomposing meat, cheese, and cream as a result of chemical change in the material, the bacteria causing the change. Then they were called *toxalbumins*, and were supposed to belong to an albumin series; but when the bacteria are grown in non-albuminous media, the toxins correspond more in their chemical composition to a *ferment*, and therefore it is supposed that the albumin part of the toxin is furnished by the blood or albuminous media in which it is formed. The term *toxin* is to be preferred in speaking of bacterial poisons.

Toxins may be of two sorts: (a) Chiefly within the bodies of the bacteria, so that they are set free by the disintegration of the organisms. This group comprises most of the pathogenic bacteria and must be combated by the use of *antibacterial* serums. (b) The poisons seem to be excreted by the bacteria and are found in the surrounding medium. *Antitoxic* serums are applicable to this group, which includes the bacilli of diphtheria and tetanus. Welch has suggested that even bacteria which do not appear to form toxins in artificial cultures may do so in the human body. In the effort to adapt themselves to their environment and resist the hostile agencies of the body they produce the poisons we call toxins. (For method of production of an antitoxin, see article on Diphtheria.)

Toxins are not stable; they are uncrystallizable, soluble in water; they are allied to albumose in that they are precipitated by alcohol and ammonium sulphate.

Aggressin.—A name given to a form of toxin developed in the animal body that has the power of rendering a germ more active (aggressive). If such a toxin is obtained from an experiment animal and added to a growth of bacteria, it makes that culture particularly virulent. Aggressins are supposed to have a paralyzing influence on the phagocytes.

CHAPTER XIV

IMMUNITY

Immunity, as distinguished from *susceptibility*, is merely a relative term, as no animal is absolutely immune under all conditions. It is merely less susceptible, and some animals are by nature or can by artificial means be rendered so slightly susceptible that to all practical purposes they are immune—that is, capable of resisting an attack of the particular disease against which they are said to be immune.

Natural Immunity.—The goat and dog are considered naturally immune to tuberculosis. Algerian sheep are resistant to anthrax; other varieties are susceptible.

The field-mouse is susceptible to glanders; the white mouse is ordinarily immune. House mice are susceptible to mouse septicemia; field-mice are immune.

Acquired Immunity.—Immunity can be acquired in many ways. Active and passive immunity are varieties.

Active immunity can be acquired from an *attack of the disease*; such infectious diseases as measles, scarlatina, and whooping-cough usually confer immunity from future attacks. Some diseases render the individual immune for only a short period.

Immunity from Inoculation with Attenuated or Weakened Cultures of Bacteria.—*Vaccination* is an example. Haffkine's cholera vaccines and Pasteur's vaccines of anthrax and chicken cholera are likewise examples of this method.

Attenuation is produced as follows: Successive cultivation in *artificial media* destroys the virulence of bacteria. Old cultures are less virulent than fresh ones. Virulence is lessened by passing the cultures through animals that are less susceptible or entirely immune. The cautious use of chemicals and sunlight lessens virulence. Heat is an effective agent. An anthrax culture exposed to a temperature of 42.6° C. for twenty days will prove destructive only to animals no larger than mice. Prolonged exposure to oxygen weakens the germs.

Immunity Through Inoculations of Small Doses of Very Virulent Microorganisms.—A graduated resistance to the disease is reached somewhat after nature's method. By successive inoculations with increased doses of the virus an immunity is often reached sufficient to withstand ten times the lethal dose. A poison-habit is thus acquired.

Increased virulence is produced as follows: The cultures may be greatly increased in virulence by successive cultivation through animals, and gradually changing from smaller animals to larger, until an amount of the culture that, at the outset, would not destroy a guinea-pig, becomes finally virulent for chickens and dogs.

Immunity Through Injections of the Sterilized Products of Bacteria.—Cultures sterilized by heat or filtration through germ-filters still contain the chemical products of bacteria—the toxins; and when these are injected in gradually increased doses, the same immunity is obtained as with the bacteria themselves.

Passive Immunity.—The blood-serum and tissues generally of animals rendered immune in the ways described above, when injected into susceptible animals, render them immune against the *same infection*. This has been called *passive immunity*, but there is no strong reason why this term should be used. The blood-serum of immune animals is simply another means for immunization. It is less permanent than the other forms of immunization, but it appears very soon after the injection, and in a modified form has a curative action even when the symptoms of the infection are already present in the system.

Inherited Immunity.—An immunity to disease *acquired* during the lifetime of the parents is probably never transmitted to the offspring, though the mother may transmit a temporary immunity to the child in utero, or the child itself may have been subjected to the infection at the same time with its mother. But this cannot be called inherited.

Theories of Immunity.—Several older theories only need to be mentioned, as they are no longer tenable. They are the exhaustion theory of Pasteur, the retention theory, and the

humeral theory. At present, modifications of Metchnikoff's phagocytic theory and Ehrlich's side-chain theory seem the most plausible.

Phagocytic or Cellular Theory.—Metchnikoff elaborated this after his study on inflammation. Phagocytosis occurs in animals when subjected to the action of an irritant. The leukocytes are attracted to the injured spot and envelop the irritating substance, be it bacteria or dead matter. The theory given out at first was that if the leukocytes conquer the bacteria, immunity results; if the bacteria eat up the leukocytes, disease occurs. Modified to suit other conditions, as, for instance, the germicidal properties of serum freed from its cellular elements, Metchnikoff states that at times *phagolysis*—that is, breaking up or solution of the phagocytes—takes place, and the fluids in which these cells are dissolved become charged with the powers originally present in the phagocytes. *Chemotaxis* is the term applied to the attraction of bacteria for the leukocytes, and is supposed to be chemical in its nature. The phagocytic cells comprise: (a) The polymorphonuclear leukocytes of the blood, termed *microphages*, and (b) a group called *macrophages*, which includes all other cells having phagocytic properties, such as leukocytes other than the polymorphonuclears, endothelial cells, and connective-tissue corpuscles. When these cells are injured, they set free their digestive ferments, known as *microcytases* and *macrocytases* respectively, which correspond to the alexins of Ehrlich.

Ehrlich's Side-chain Theory.—This derives its name from the fact that it presents an analogy to what happens in the benzol ring of organic chemistry when its replaceable atoms of hydrogen are substituted by "side chains" of more or less complex nature. The molecule of protoplasm is supposed to consist of a central atom group provided with a large number of side chains which subserve the vital processes of the molecule by combining with other organic molecules. These side chains are called receptors, and are of many different kinds, so as to fit them for combination with many different varieties of extraneous groups. Bacterial toxins contain two groups: (1) The

haptophores, by which the toxin molecule can become joined to the cell; (2) the toxophores, by virtue of which it can attack the protoplasm after having been fixed to it by the haptophore. If the attack on the molecule is not too severe, this is stimulated into overactivity and throws out an abnormal number of receptors, some of which (the haptins) become detached and are capable of uniting with free haptophores and preventing their combination with the protoplasm of the molecule. In other words, they represent the antitoxin.

Hemolytic Serum.—The blood-serum of some animals has the power of destroying or dissolving the red corpuscles of another animal of different species. This power can be increased by repeated injections of blood-corpuscles, and is allied to the bactericidal power.

Bacteriolysis is the destruction of the bacterial cells by the blood-serum, and is probably effected in a somewhat different manner. Antibacterial serums are effective through the combined activities of a destructive element, the “complement” (alexin or cytase), and an “immune body” (amboceptor), which serves the function of joining the complement to the bacterial molecule. These two bodies differ markedly in their properties—for example, the complement is destroyed at 60° C., while the immune body is very resistant.

It is not stated what cells are the sources of these various antibodies, but probably any cell capable of being attacked by a toxin is also capable of responding by the production of anti-substances.

Lysins.—The substances producing destruction of bacteria are called lysins. Normal blood-serum is bacteriolytic to a slight degree, but during infection produces lysins specific for the germ in question.

Agglutinins.—These are bodies formed in the blood-serum in response to the stimulation of certain bacteria, such as the typhoid bacillus, *Bacillus coli communis*, *Micrococcus melitensis*, the bacillus of dysentery, the cholera spirillum, etc. When such a serum is added to cultures of the particular organism concerned, the bacteria become clumped in motionless

masses. A modified form of agglutination, in which long strings of bacteria are formed, is known as the "thread" reaction.

Agglutinins are of different kinds, and they are not an indication of immunity. A serum may give a strong agglutinating reaction, but have little or no bactericidal power. The power varies from day to day—it is strongest in the blood-serum, but is found in other fluids of the body.

Agglutinogen is the name given to the substance on the bacteria, while agglutinin is the term restricted to the substance in the serum.

Precipitins.—Animals immunized to certain bacteria or to albumins of different sorts form bodies which cause the blood-serum to give a precipitate when added to cultures of these organisms or fluids containing the specific albumin. The phenomenon has found forensic application in the identification of blood-stains.

Opsonins.—Much work has been done in the last few years to harmonize the various theories on immunity, and a new one, advanced by Wright and Douglas in 1905, seems to have much in its favor.

Briefly stated, the leukocytes have no power in themselves to act upon bacteria, but derive this property from the blood-serum. In normal blood-serum Wright maintains there is a body which he calls "opsonin," which becomes fixed to the bacterial cells and makes them subject to phagocytosis. This power of the serum is greater the more immune the animal furnishing it is.

It can be increased by whatever increases immunity; in other words, it is coincident with immunity.

History.—*Denys and Leclif* (1895) proved that the serum of a vaccinated rabbit altered bacteria so as to permit their ingestion by leukocytes.

Mennes (1897) showed that this modification resides in the serum and not in the leukocytes.

Leishman (1902) devised a method of estimating the phagocytic power of serums.

Wright (1902) showed that sterile cultures of certain micrococci given in small doses raised the agglutinating power of the blood to that particular organism. Wright (1903) using Leishman's method, found that the phagocytic index is raised by the injection of bacterial products.

Wright and Douglas (1903) gave the name of *opsonin* (from *opsono*, I prepare the food for) to this element in the blood, arguing that in some way the bacteria are acted upon by this substance, making them more readily digested.

Opsonic Index.—The degree of immunity of a given blood-serum, as compared with that taken from a healthy individual, considered as a unit, is called the *index*.

The activity of the leukocytes toward an emulsion of bacteria is noted first when washed with normal serum, and then when washed with serum from an affected person. If, in the one instance, in 50 cells an average of 3 is obtained, and, in the other instance, the average is only $1\frac{1}{2}$, the index is then said to be 0.5, or one-half of the normal. This index is measured from day to day with the course of the disease and treatment, and charted just as the temperature is.

A *negative phase*, that is, a decreased opsonic index, often follows the use of bacterial products; then comes a *positive phase*, or a rise in phagocytosis. Injections of vaccines are harmful if repeated before the negative phase has worn off.

Summary of Immunity Theories.—At this writing the following ideas concerning immunity seem to be accepted:

The blood-serum contains substances—opsonins—which envelop or attach themselves to bacterial cells, rendering them fit for leukocytic digestion—phagocytosis. The growth of bacteria in the body stimulates the production of antibodies,—antitoxins,—and the activity of the blood-cells likewise calls forth an increased activity of the germ and an increase in toxin. The theory of Ehrlich, or chemical explanation of the action of antitoxin, is still a subject of controversy.

CHAPTER XV

EXPERIMENTS UPON ANIMALS

THE smaller rodents and birds are the ones usually employed for inoculation, as rabbits, guinea-pigs, rats, mice, pigeons, and chickens, sometimes monkeys. These are preferred, because easily acted upon by the various bacteria, readily obtained, and not expensive.

The white mouse is very prolific and easily kept, and is therefore a favorite animal for experiment. It lives well upon a little moistened bread. A small box, perforated with holes, is filled partly with sawdust, and in this ten to twelve mice can be kept. When the female becomes pregnant, she should be removed to a glass jar until the young have opened their eyes, because the males, which have not been *raised together*, are apt to attack each other.

Guinea-pigs.—When guinea-pigs have plenty of light and air, they multiply rapidly. Therefore it is best to have them in some large stall or inclosure. They can be fed upon all sorts of vegetables and grasses, and require but little attention.

Methods of Inoculation.—*I. Inhalation.*—Imitating the natural infection, either by loading an atmosphere with the germs in question or by administering them with a spray.

II. Through skin or mucous membrane.

III. With the food.

Method of Cutaneous Inoculation.—The ear of mice is best suited for this procedure. A small abrasion made with the point of a lancet or needle, which has been dipped in the virus. The animal is then separated from the rest and placed in a glass jar, which is partly filled with sawdust and covered with a piece of wire gauze.

Subcutaneous.—The root of the tail of mice is used for this purpose. The hair around the root of the tail is clipped off, and with a pair of scissors a very small pocket is made in the subcutaneous connective tissue, not wounding the animal any

more than absolutely necessary, avoiding much blood. The material is placed upon a platinum needle and introduced into the pocket; *solid bodies*, with a forceps.

To hold the mouse still while the operation is going on a little cone made of metal is used. The mouse just fits in here. There is a slit along the top in which the tail can be fastened, and thus the animal is secure and immobile.

Intravenous Injections.—Rabbits are very easily injected through the veins. Mice are too small.

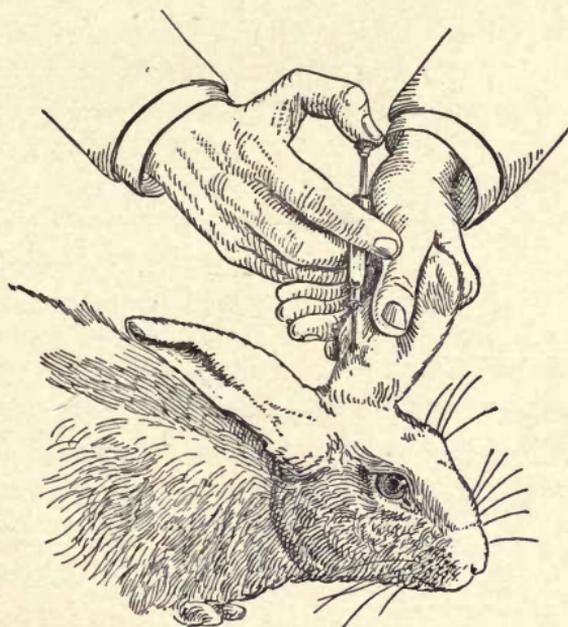


Fig. 45.—Method of making an intravenous injection into a rabbit. Observe that the needle enters the posterior vein from the hairy surface (McFarland).

The ear of the rabbit is usually taken. It is first washed with 1 : 2000 bichlorid, which not only disinfects, but also makes the vessels appear more distinct. The base of the ear is compressed to swell the veins. Then a syringe, like the one used for the injection of "tuberculin," a Koch syringe, which can be easily sterilized, is filled with the desired amount of virus and slowly injected into any one of the more prominent veins present (Fig. 45).

Intraperitoneal Injection.—This is used with guinea-pigs mostly. The abdominal wall is pinched up through its entire thickness, and the needle of the syringe thrust directly through, so that it appears on the other side, then the fold let go, the needle withdrawn just far enough so as to be within the cavity.

Inoculation in the Eye.—The anterior chamber and the cornea are the two places used. The rabbit is fixed upon a board, the eyelids held apart and head held still by an assistant. A small cut is made in the cornea, a few drops of cocain having first been introduced in the eye. The material is passed through the opening with a small forceps, and with a few strokes of a spoon it is pushed in the anterior chamber.

For the cornea a few scratches made in the corneal tissue will suffice; the material is then gently rubbed in.

Inoculation of the Cerebral Membranes.—The skin and aponeurosis cut through where the skull is the thinnest. Then the bone carefully trephined, and the dura exposed. In *rabies* inoculation, the syringe containing the hydrophobic virus pierces the dura and arachnoid, and the virus is discharged beneath the latter.

Intratracheal.—The bacteria can be introduced directly into the trachea, thus coming in contact with the lungs.

Intraduodenal.—Cholera germs are injected into the intestines after they have been exposed, by carefully opening the abdomen. This is done in order to avoid the action of the gastric juice.

Celloidin sacs of small size are sometimes used to introduce living cultures of bacteria into the bodies of animals without their coming into direct contact with the tissues.

Obtaining Material from Infected Animals.—The animal should be skinned, or the hairs plucked out, before it is washed—at least the portion where the incision is to be made. Then the entire body is washed in sublimate. Two sets of instruments are required—one for coarser and one for finer work: the one sterilized in the flame; the other, to prevent being damaged, heated in a hot-air oven.

The animal, the mouse, for example, is stretched upon a

board, a nail or pin through each leg, and the head fixed with a pin through the nose. The skin is dissected away from the belly without exposing the intestines. Then the ribs being laid bare, the sternum is lifted up, and the pericardium exposed. A platinum needle dipped into the heart after the pericardium has been slit will give sufficient material for starting a culture. If the other organs are to be examined, further dissection is made. If the intestines were first to be looked at, they would be laid bare first.

In this manner material is obtained and the results of inoculation noted.

Frequent sterilization of the instruments is desirable.

Koch's Rules in Regard to Bacterial Cause of Disease.—

Before a microbe can be said to be the cause of a disease, it must—

First, be found in the tissue or secretions of the animal suffering from, or dead with, the disease.

Second, it must be cultivated outside of the body on artificial media.

Third, a culture so obtained must produce the disease in question when it is introduced into the body of a healthy animal.

Fourth, the same germ must then again be found in the animal so inoculated.

CHAPTER XVI

OPSONIC TECHNIC

Method of Counting Bacteria in a Culture.—In the use of protective vaccines, a method of counting the bacteria in a given amount of serum or emulsion has come into practice, especially in the opsonic index treatment.

First, the number of red cells in a cubic millimeter of blood is measured; then, in a capillary tube (Wright pipet), blood

is sucked up to a certain mark, and, with an air-bubble between, a diluted suspension of bacterial culture is measured into the same tube. From this mixture, one drop is blown out on to a slide and stained according to Leishman's method, and the bacteria compared to the blood-cells. If the observer's blood contained 5,000,000 red cells in a cubic millimeter, and with a 1 : 3 dilution of the bacteria, the bacteria are equal in number to the red cells, in the undiluted culture each cubic millimeter would contain 15,000,000 bacterial cells.

Method of Measuring Phagocytosis—the Opsonic Technic.—*First, Preparation of Bacterial Emulsion.*—The culture

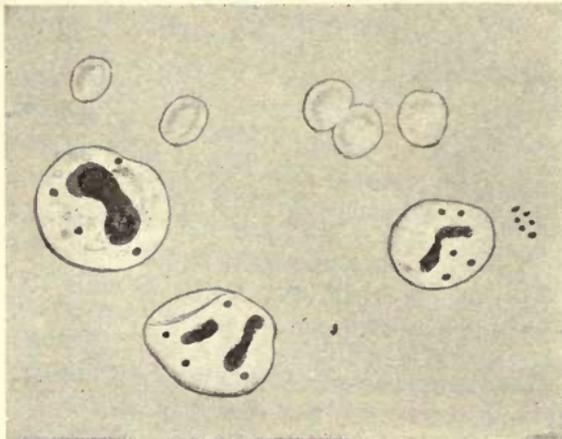


Fig. 46.—Diagram of phagocytosis. Staphylococci in polynuclear leukocytes.

from an agar slant is rubbed in a watch-crystal with 0.85 per cent. saline solution, and the mixture centrifugalized to sediment any masses undissolved. The emulsion should then be just very slightly cloudy, and will contain about 10,000,000,000 bacteria per cubic centimeter.

Second, Preparation of Leukocytes.—Healthy blood-cells are used—preferably the observer's. At least ten drops of blood are mixed in a test-tube containing 3 c.c. warm normal salt solution, to which 1.5 per cent. sodium citrate has been added; this is then centrifuged, the supernatant fluid removed down to the layer of leukocytes, and an equal quantity of normal salt

solution added and again the mixture centrifuged, the fluid removed, leaving the leukocytes and red cells, which is known as the leukocyte emulsion or "*washed leukocytes.*"

Third, Preparation of the Serums.—Small capsules made from glass tubing (see Fig. 47) are used to obtain the blood from a normal or healthy person; the blood by attraction is made to fill a capsule, the end is sealed with wax, and the corpuscles separated by the centrifuge. A capsule with blood from an infected person is obtained, and the serum separated in the same way.

Fourth, Mixture of the Emulsion, the Leukocytes, and the Serum.—This is performed by drawing into a pipet similar portions of the emulsion, the leukocytes, and the serum, mixing

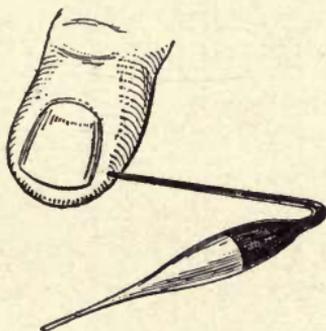


Fig. 47.—Wright's blood-capsule. Illustration shows technic of filling same.

a drop of each on a slide. The mixture is then drawn up again into the tube, the end of which is now sealed and the tube placed in an incubator at 37° C. for fifteen minutes. The end is broken, and a drop of the mixture is distributed evenly on a glass slide and stained. The same procedure is carried on with the serum of the infected person, but using the normal leukocytes.

Under the microscope the number of bacteria in the protoplasm of at least fifty polynuclear cells are counted (see Fig. 46) in the mixture with the normal serum and in the mixture with the infected serum, and an average for each obtained; the proportion that the diseased bears to the normal is the *opsonic index*.

In this work the following special apparatus are required:

1. A *Wright's tube* for collecting the blood.
2. Four c.c. test-tubes 7 cm. long and 9 mm. in diameter, to hold the washed leukocytes.

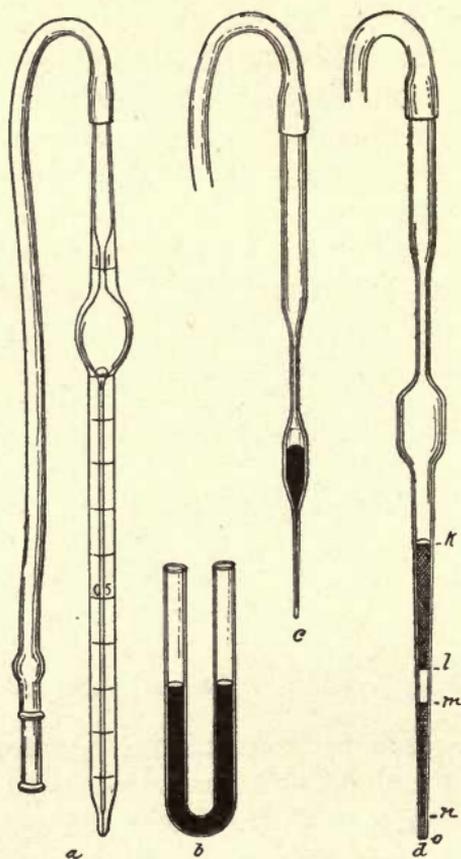


Fig. 48.—Tubes utilized in testing agglutinating and sedimenting properties of serum: *a*, Leukocytometer pipet; *b*, V-shaped tube; *c*, capillary pipet; *d*, Wright's sedimentation tube.

3. Suction pipets, either Gowers' graduated pipet, as used in blood examinations, or a special device of Wright's (see *d*, Fig. 48).

PART II

SPECIAL BACTERIOLOGY

CHAPTER XVII

NON-PATHOGENIC BACTERIA

Special Bacteriology.—Under this head the chief characteristics of individual bacteria will be detailed, *pathogenic* and *non-pathogenic* being the main divisions. The division is not a strict one, under certain conditions bacteria ordinarily non-pathogenic may become pathogenic.

Non-pathogenic Bacteria.—The list of non-pathogenic bacteria is a long one, and is being added to continually.

Bacillus Prodigiosus (Ehrenberg).—This bacillus, formerly called micrococcus, is very common, and one of the first noticed, because of the brilliant red pigment it forms on vegetables and starchy substances. “The bleeding host” miracles are said to have been due to it.

Form.—Short rods, often in filaments, *without spores*.

Immobile.—Has no automatic movements.

Facultative anaërobic, that is, it can grow without air; but the pigment requires oxygen to show itself.

Growth.—*Gelatin*. Liquefy rapidly.

Colonies.—At first white, round points with smooth edge, appearing brown under microscope, but soon changing to red.

Stab-cultures.—Red pigment develops on the surface, the growth occurring all along the line.

Potato is well suited to the growth, the pigment developing after twelve hours. *Agar* and *blood-serum* well suited for growth.

Temperature.—Grows best at 25° C.

Varieties.—By exposure to heat of brood-oven during several generations the power to produce pigment can be temporarily abolished.

The Pigment.—A pigment-forming body is created by the bacillus, and the action of oxygen upon it produces the color. It is insoluble in water, slightly soluble in alcohol and ether; acids fade it, alkalis restore the color. The pigment resembles fuchsin, presenting the same metallic luster.

Gases.—A trimethylamin odor arises from all cultures.

Stain.—With all dyes in the ordinary way.

Bacillus Indicus (Koch).—*Synonym.*—*Micrococcus Indicus.*

Origin.—Found in the stomach of an Indian ape.

Form.—Short rods with rounded ends. No spores. *Automatic movements* present; *facultative anaërobin.*

Growth.—*Gelatin.*—Liquefy rapidly.

Colonies.—Round or oval granular margins; brilliant *red pigment.*

Stab-cultures.—The pigment shows itself on the surface. Grows well on all media.

Temperature.—Grows best at 35° C.

Action on Animals.—In very large quantities, if injected into the blood, a severe and fatal gastro-enteritis can be produced.

Stain.—Takes all dyes.

Bacillus Mesentericus Vulgatus.—The common potato bacillus of *Flügge* (Fig. 49).

Habitat.—Surface of the soil, on potatoes, and in milk.

Form.—Small thick rods with rounded ends, often in pairs.

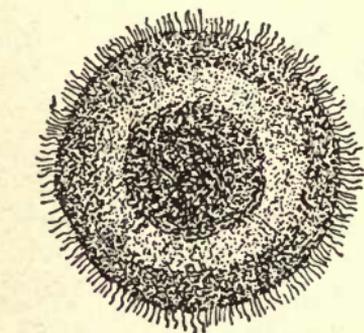


Fig. 49.—Colony of *Bacillus mesentericus vulgatus.*

Properties.—Very motile; produces abundant spores; liquefy gelatin; diastatic action.

Growth.—Rapid.

Plate Colonies.—Round, with transparent center at first, then

becoming opaque. The border is ciliated; little projections evenly arranged.

Potato.—A white covering at first, which then changes to a rough brown skin; the skin can be detached in long threads.

Temperature.—Spores at ordinary temperature.

Spores.—Are very resistant; are colored in the manner described in first part of the book for spores in general.

Bacillus Megaterium (de Bary) (Fig. 50).—*Origin*.—Found on cooked cabbage and garden-soil.

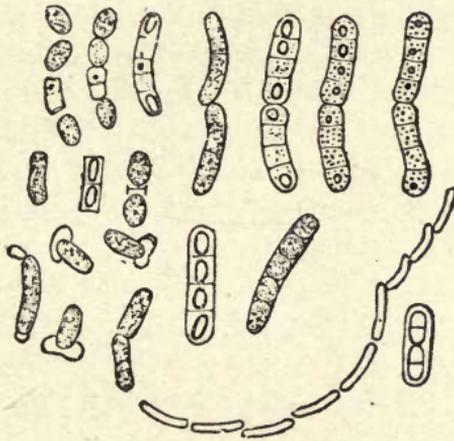


Fig. 50.—*Bacillus megaterium*, with spores.

Form.—Large rods, four times as long as they are broad, 2.5μ . Thick rounded ends. Chains with ten or more members often formed; granular cell contents.

Properties.—Abundant spore formation; very slow movement; slowly dissolves gelatin.

Growth.—Strongly aërobic; grows quickly and best at a temperature of 20° C.

Plate Colonies.—Small, round, yellow points in the depth of the gelatin. Under microscope, irregular masses.

Stab-culture.—Funnel-shaped from above downward.

Potato.—Thick growth with abundance of spores.

Bacillus Ramosus.—*Synonyms*.—*Bacillus mycoides* (Flügge); Wurzel or root-bacillus.

Origin.—In the upper layers of garden or farm grounds and in water.

Form.—Short rods, with rounded ends, about three times as long as they are thick; often in long threads and chains.

Properties.—Large, shining, oval spores; a slight movement; *liquefy gelatin.*

Growth.—At ordinary temperature, with plentiful supply of air.

Plate Colonies.—Look like roots of an old tree gnarled together, radiating from a common center. Liquid on surface.

Stab-culture.—Soon a growth occurs along the needle-track, and the whole resembles a pine tree turned upside down. The gelatin then becomes liquid, a thin skin floating on top, and small flakes lying at the bottom.

Stroke-culture.—Feathery resemblance is produced.

Staining.—Spores stain readily with the ordinary spore stain.

Bacterium Zopfii (Kurth).—*Origin.*—Intestines of a fowl.

Form.—Short thick rods forming long threads coiled up, which finally break up into spores, which were once thought to be micrococci.

Properties.—Very motile; does not dissolve or liquefy gelatin.

Growth.—In thirty hours abundant growth; *aërobic*; grows best at 20° C.

Plates.—Small white points which form the center of a very fine netting. With high power this netting is found composed of bacilli in coils, like braids of hair.

Excellent impress or “Klatsch” preparations are obtained from these colonies.

Staining.—Ordinary dyes.

Bacillus Subtilis (Hay Bacillus) (Ehrenberg).—*Origin.*—Hay infusions; found also in air, water, soil, feces, and putrefying liquids. Very common, often contaminates cultures.

Form.—Large rods, three times as long as broad; slight roundness of ends; seldom found singly; usually in long threads. *Flagella* are found on the ends. *Spores* of oval shape, strongly shining, very resistant.

Properties.—Very motile; dissolves gelatin.

Growth.—Rapid; strongly aërobic.

Plate.—Round, gray colonies with depressed white center. Under microscope the center yellow; the periphery like a wreath, with tiny little rays projecting; very characteristic.

Potato.—A thick moist skin forms in twenty-four hours.

Staining.—Rods, ordinary stain; spores, spore stain.

It is easily obtained by covering finely cut hay with distilled water, and boiling a quarter of an hour. Set aside forty-eight hours. A thick scum will show itself on the surface, composed of the subtilis bacilli, whose spores alone have survived the heat.

Bacillus Spinosus (Lüderitz).—Called spinosus because small spine-like processes are formed by the colonies.

Origin.—In the juices of the body of a mouse and guinea-pig which were inoculated with garden-earth.

Form.—Large rods, straight, some slightly bent, ends rounded; often in long threads.

Properties.—Large spores, the bacillus enlarging to allow the spores to develop; very motile; gelatin slowly liquefied. A gas is formed in the culture having an odor like Swiss cheese.

Growth.—The growth occurs at 20° C. temperature only when the oxygen is excluded. Very strongly anaërobic. Glucose added to the gelatin (1 to 2 per cent.) increases growth.

Colonies in *roll cultures* and *high stab-cultures* appear as little spheres surrounded by a zone of liquefied gelatin. In the deeper growths thorn-like projections or spines develop, proceeding from a gray-colored center.

Staining.—With ordinary methods.

Some Bacteria Found in Milk.—Bacillus Acidi Lactici (Hüppe).—Belongs to the same group as the *Bacillus coli communis*. (See p. 137.)

Origin.—In sour milk.

Form.—Short thick rods, nearly as broad as they are long, usually in pairs.

Properties.—Immotile. Spores, large shining ones. Does not liquefy gelatin. Breaks up the sugar of milk into lactic acid and carbonic acid gas, the casein being thereby precipitated.

Growth.—Slow; is facultative anaërobic. Grows at 10° C.

Plate Colonies.—First small white points, which soon look like porcelain, glistening. Under microscope the surface colonies resemble leaves spread out.

Stab-culture.—A thick dry crust with cracks in it forms on the surface after a couple of weeks.

Attenuation.—If grown through successive generations, they lose the power to produce fermentation. Several other bacteria will give rise to lactic-acid fermentation; but this especial one is almost constantly found and is very widespread.

In milk it first produces acidity, then precipitation of casein, and, finally, formation of gases.

A bacillus described by Grotenfeldt, and called *Bacterium acidi lactici*, forms alcohol in the milk. It was found in milk in Bavaria.

Boas-Oppler Bacillus.—Also known as the *Bacillus geniculatus*. Owing to the faculty possessed by this organism of growing in the presence of amounts of lactic acid sufficient to check the development of all other lactic-acid formers, it usually predominates in stomach-contents containing large amounts of this substance. The parent type is composed of short rods, but in the presence of considerable amounts of lactic acid these change to a longer form, which occurs singly or in long chains. It is stained brown by Gram's iodine solution. The bacillus affords confirmatory evidence of the presence of a new growth, though it may occur in benign conditions.

Bacillus Butyricus (Hüppe).—This bacillus causes butyric-acid fermentation.

Origin.—Found in milk.

Form.—Short and long thin rods with rounded ends; large oval spores, seldom forming threads.

Properties.—Very motile; liquefies gelatin rapidly; produces gases resembling butyric acid in odor. In milk it coagulates the casein, decomposes it, forming peptones and ammonia, with a bitter taste, and butyric-acid fermentation. An alkaline reaction.

Growth.—Quickly, at 35° to 40° C., with oxygen. Spores very resistant.

Colonies.—*Plate.*—Small yellow points which soon run together, becoming indistinguishable.

Stab-culture.—A small yellow skin forms on the surface with delicate wrinkles; cloudy masses in the liquefied portion.

Staining.—With ordinary stains.

Bacillus Amylobacter (Van Tiegham); synonyms **Clostridium Butyricum (Prazmowsky)**; **Vibriion Butyrique of Pasteur (Fig. 51).**—*Origin.*—Found in putrefying plant-infusions, in fossils, and conifera of the coal period.

Form.—Large, thick rods, with rounded ends, often found in chains. A large glancing spore at one end, the bacillus becoming spindle-shaped in order to allow the spore to grow; hence the name, clostridium.

Properties.—Very motile; gases arise with butyric smell. In solutions of sugars, lactates, and cellulose-containing plants and vegetables, it gives rise to decompositions in which butyric acid is often formed. Casein is also dissolved.

Like granulose, a watery solution of iodine will color blue some portions of the bacillus; therefore it has been called *amylobacter*.

Growth.—It is strongly anaërobic, and has not yet been satisfactorily cultivated.

Bacillus Lactis Cyanogenus (Bacterium Syncyanum) (Hüppe).—*Origin.*—Found in blue milk.

Form.—Small narrow rods about three times longer than they are broad; usually found in pairs. The ends are rounded.

Properties.—They are very motile; do not liquefy gelatin; form spores usually in one end. A bluish-gray pigment is formed outside of the cell, around the medium. The less alkaline the media, the deeper the color. It does not act upon the milk otherwise than to color it blue.

Growth.—Grows rapidly, requiring oxygen. *Colonies on plate.* Depressed center, surrounded by ring of porcelain-like bluish growth. Dark-brown appearance under microscope.

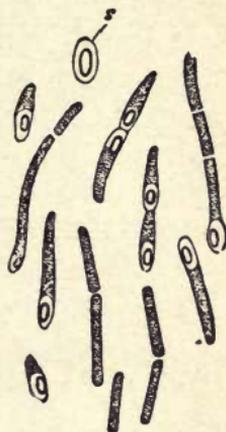


Fig. 51.—*Bacillus amylobacter*.

Stab-culture.—Grows mainly on surface; a nail-like growth. The surrounding gelatin becomes colored brown.

Potato.—The surface covered with a dirty blue scum.

Attenuation.—After prolonged artificial cultivation loses the power to produce pigment.

Staining.—By ordinary methods.

Bacillus Lactis Erythrogenes (Bacillus of Red Milk) (Hüppe and Grotenfeldt).—*Origin.*—Found in red milk and in the feces of a child.

Form.—Short rods, often in long filaments, without spores.

Properties.—Does not possess self-movement. Forms a nauseating odor; liquefies gelatin. Produces a yellow pigment which can be seen in the dark, and a red pigment in alkaline media, away from the light. In milk it produces the yellow cream on top of the blood-red serum, or fluid in the center, and at the bottom the precipitated casein.

Growth.—Grows rapidly in bouillon and on potatoes; slower on the other media. *Plates.* A cup-like depression in the center of the colony, with a pink coloration around it, the colony itself being slightly yellow.

Stab-culture.—The growth mostly on surface. The gelatin afterward colored red and liquefied.

Potato.—A golden-yellow pigment formed at 37° C. after six days.

Examination of Milk in Stained Specimen.—A drop of milk diluted with a drop of distilled water is dried on the cover-glass and fixed by heat. Chloroform methylene blue, prepared by mixing 12 to 15 drops of saturated alcoholic solution of methylene blue with 3 or 4 c.c. of chloroform, is used for staining. The chloroform is then evaporated by exposing the specimen for a few minutes to the air. Bacteria blue; rest of field, unstained.

Another method is to mix a drop of milk with two or three drops of a 1 per cent. solution of sodium carbonate on a cover-glass. Saponification of the fat occurs on heating the mixture to evaporation. The preparation is then stained in the ordinary manner.

Some Non-pathogenic Bacteria Found in Water.—The bacteria found are very often given to producing pigments or phosphorescence, and are a great number; they frequently give rise to foul gases and, for the most part, liquefy gelatin rapidly.

Bacillus Violaceus.—*Origin.*—Water.

Form.—A slender rod with rounded ends, three times as long as it is broad, often in threads; middle-sized spores.

Properties.—Very motile; forms a violet-blue pigment, which is soluble in alcohol, and depends upon oxygen for its growth. Rapidly liquefies gelatin, but not agar.

Growth.—Grows fairly quick, is facultative anaërobic.

Cultures on Plate.—At first the colonies look like inclosed air-bubbles. Low power shows irregular masses, with a center containing the pigment and a hairy-like periphery.

Stab-culture.—Cone-like liquefaction containing air, and the pigment, in separated granules, lying toward the bottom.

Stroke Culture on Agar.—A violet, ink-like covering which remains for years.

Bacillus Cœruleus (Smith).—*Origin.*—Schuylkill water.

Form.—Very thin rods; 2.5μ long, 0.5μ wide; often in threads; spores were not found.

Properties.—Liquefies gelatin; produces a very deep-blue pigment.

Growth.—Slowly, with oxygen, at ordinary temperature.

Plate.—Round colonies on the surface of bluish color.

Stab-cultures.—A cup-shaped liquefaction along the needle-thrust, with a sparse growth, the liquefied portion appearing blue.

Fluorescent Bacteria.—Several kinds present in water.

Bacillus Erythrosporus (Eidam).—*Origin.*—Drinking-water and putrefying albuminous solutions.

Form.—Slender rods, often in short threads, with spores of oval shape, and appearing as if stained with fuchsin.

Properties.—Motile; does not dissolve gelatin; produces a greenish, fluorescent pigment, which appears yellow in reflected light, but green on transmitted light.

Growth.—Somewhat quickly; facultative anaërobic; growth only at ordinary temperatures.

Plates.—White colonies, with greenish-yellow fluorescence around each colony. Under microscope the periphery appears radiated.

Stab-cultures.—Good growth along the needle-thrust; the whole gelatin gives out the fluorescence.

Bacillus Fluorescens Liquefaciens.—*Origin*.—Water and from conjunctival sac.

Form.—Very fine little rods; no spores.

Properties.—Motile; forms a greenish-yellow, fluorescent pigment; liquefies gelatin.

Growth.—Rapid at ordinary temperature and strongly aërobic.

Plates.—Round colonies, cup-shaped depressions, the solid gelatin that remains becoming colored with greenish-gelbow fluorescence.

Stab-culture.—On the surface, air-bubble depressions; the white colonies in the bottom of these depressions and the solid gelatin around the inoculation shining with the fluorescence.

Phosphorescent Bacteria.—Are found usually in sea-water or upon objects living in the sea.

Bacillus Phosphorescens Indicus (Fischer).—*Origin*.—Tropical waters.

Form.—Thick rods, with rounded ends, sometimes forming long threads.

Properties.—Very motile; liquefying gelatin at a temperature of 25° to 30° C., with oxygen and a little moisture, and in the dark, a peculiar electric-blue phosphorescence develops.

Growth.—Slowly; must have oxygen; does not grow under 10° C. or over 50° C.

Plates.—Little round, gray points, which under low power appear as green colonies with reddish tinge around them.

Cooked fish, when smeared upon the surface with a little of the culture, show the phosphorescence most marked. Grows well on *potatoes* and *blood-serum*.

Bacillus Phosphorescens Indigenus (Fischer).—*Origin*.—Waters in the northern part of Germany. It differs from the

Indian bacillus in that it grows at a temperature of 5° C., and does not develop upon potatoes or blood-serum.

Bacillus Phosphorescens Gelidus (Förster).—*Origin.*—Surfaces of salt-water fish.

Form.—Short, thick rods, looking oval sometimes; zoöglea are often formed.

Properties.—Motile; does not liquefy gelatin; a beautiful phosphorescence from the surface of fish; it can be photographed by its own light.

Colonies.—Grows best between 10° and 20° C.; grows slowly, and mostly on the surface. The material must contain salt. A bouillon made with sea-water or 3 to 4 per cent. common salt will suffice. The colonies appear as those of the *Phosphorescens Indicus*.

Fresh herring laid between two plates will often show phosphorescence in twenty-four hours.

Three varieties require *glucose* in the culture before they give out any glow. They are *Bacterium Pflügeri*, *Bacterium Fischeri*, and *Bacterium Balticum*. They do not dissolve gelatin.

Several very indistinct species, found in waters from factories and in some of the mineral waters, deserve yet to be mentioned. They have been given various names by observers; and a new classification created. Such are the *crenothrix*, *cladothrix*, and *beggiatoa*, which belong to the "higher bacteria."

Crenothrix Kühniana (Rabenhorst).—Long filaments joined at one end; little rod-like bodies form in the filaments, and these break up into spores.

Zoöglea are also formed by means of spores, and these can become so thick as to plug up pipes and carriers of water. They are not injurious to health.

Cladothrix Dichotoma (Cohn).—Very common in dirty waters. The filaments branch out at acute angles, otherwise resembling the *crenothrix*; accumulations of ocher-colored slime, consisting of filaments of this organism, are found in springs and streams. (See Fig. 128.)

Leptothrix Buccalis.—In the mouth, long filaments or threads resembling bacteria are commonly found. At one end

are seen numerous cocci-like bodies, which some regard as spores. A variety of this, or a nearly allied organism, is the most frequent cause of noma or gangrenous stomatitis.

With iodine the leptothrix is colored yellow. At one time it was considered the cause of "tartar" on the teeth, and often it fills the crypts of the tonsils, forming there small masses which are difficult to remove. Miller distinguishes three varieties—*Leptothrix buccalis innominata*, *maxima*, and *gigantea*.

Beggiatoa Alba (Vancher).—The most common of this species. The distinction between this and the preceding species lies in the presence of sulphur granules contained in the structure, and hence they are often found where sulphur or sulphids exist; but where the remains of organic life are decomposing they can also be found.

Several large spirilla and vibrio live in bog and rain-water, but our space does not suffice to describe them. For the Bacteriologic Examination of Water see page 220.

Microorganisms Found in Urine.—When freshly passed, urine of a normal state contains no bacteria. By contact with the air and the urinary passages exposed to air, a great number of yeast molds and bacteria soon accumulate in the fluid. Bacteria also enter urine through the blood and during its secretion.

A number of bacteria have the property of converting urea into carbonate of ammonia.

The urine should be centrifuged and the deposit then examined. The drying and fixing must proceed very slowly, since otherwise crystals of salts will be precipitated and mar the specimen.

Bacterium Ureæ.—*Origin.*—Decomposed ammoniacal urine.

Form.—Thick, little rods, with round ends one-half as thick as they are long.

Properties.—Does not dissolve gelatin; changes urea into ammonium carbonate.

Growth.—At ordinary temperatures, very slowly. In two

days on gelatin very minute points, which in ten days have the size of a cent. The colonies grow in concentric layers.

Micrococcus Ureæ (Pasteur and Van Tiegham).—*Origin.*—Decomposed urine and in the air.

Form.—Cocci, diplococci, and streptococci.

Properties.—Decomposes urea into ammonium carbonate; does not liquefy gelatin.

Growth.—Grows rapidly, needing oxygen; can remain stationary below 0° C., growing again when a higher temperature is reached.

Colonies on Plate.—On the surface like a drop of wax.

Stab-cultures.—Looks like a very delicate thread along the needle-thrust.

Other bacteria are found in urine in various pathologic processes, such as tubercle bacilli, typhoid bacilli, gonococci, and other pyogenic organisms.

The **Urobacillus liquefaciens**, found by Schnitzler and Krogius in cystitis, is supposed to stand in close relationship to this disease.

Spirilla.—A number of non-pathogenic spirilla have been described.

Spirillum Rubrum (Esmarch).—*Origin.*—Body of a mouse dead with septicemia.

Form.—Spirals of variable length, long joints, flagella on each end; no spores.

Properties.—Does not liquefy gelatin; very motile; produces a wine-red pigment, which develops only in absence of oxygen.

Growth.—Can grow with oxygen, but is then colorless; grows very slowly; ten to twelve days before any sign; grows best at 37° C.

Gelatin Roll-cultures.—Small, round; first gray, then wine-red colonies.

Stab-cultures.—A red-colored growth along the whole line; it is deepest below, getting paler as it approaches the surface.

Spirillum Concentricum (Kitasato).—*Origin.*—Decomposed blood.

Form.—Short spirals, two or three turns, with pointed ends; it has flagella on the ends.

Properties.—Very motile; does not liquefy gelatin.

Growth.—Very slow; mostly on the surface; best at ordinary temperatures.

Plates.—A growth of rings concentrically arranged, every alternate one being transparent; the furthest one from the center possessing small projections.

Stab-cultures.—Growth mostly on the surface.

Sarcina.—*Cocci* in cubes or packets of colonies. A great number have been isolated, many producing very beautiful pigments. The majority of them found in the air.

Sarcina Lutea (Schröter).—*Origin.*—Air.

Form.—Very large cocci in pairs; tetrads and groups of tetrads.

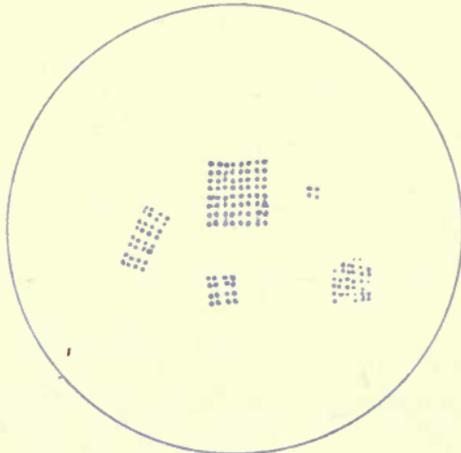


Fig. 52.—*Sarcina ventriculi* from stomach-contents ($\times 530$) (Van Valzah and Nisbet).

Properties.—Liquefies gelatin slowly; produces sulphur-yellow pigment.

Growth.—Slowly, at various temperatures; strongly aërobic.

Plates.—Small, round, yellow colonies.

Stab-cultures.—Grows more rapidly, the growth being nearly all on the surface, a few separated colonies following the needle-thrust for a short distance. *Agar*, a very beautiful yellow, along the stroked surface.

Sarcina Aurantica.—*Flava, rosea, and alba* are some of the other varieties. Many are *obtained* from beer.

Sarcina Ventriculi (Goodsir) (Fig. 52).—*Origin.*—Stomach of man and animals.

Form.—Colorless, oval cocci, in groups of eight and packets of eight.

Properties.—Does not liquefy gelatin; shows the reaction of *cellulose* to iodine.

Growth.—Rapid. At end of thirty-six hours, round, yellow colonies, from which colorless cocci and cubes are obtained.

Habitat.—They are found in many diseases of the stomach, especially when dilatation exists. Also normally; increased when fermentation occurs.

CHAPTER XVIII

PATHOGENIC BACTERIA

WE have divided this part into two portions:

- I. Bacteria which are pathogenic for man and other animals.
- II. Bacteria which do not affect man, but are pathogenic for the lower animals.

Here again it will be possible to give only the more important bacteria; there are many diseases in which microorganisms have been found, but they have not yet been proved as causative of the disease, and have also been found in other diseases. We cannot treat of them here.

Bacillus Anthracis (Rayer and Davaine).—Rayer and Davaine, in 1850, first described this bacillus; but *Pasteur*, and later *Koch*, gave it the importance it now has.

Synonyms.—Bacterie du charbon (Fr.), Milzbrand bacillus (German); bacillus of splenic fever or malignant pustule.

Origin.—In blood of anthrax-suffering animals.

Form.—Rods of variable length, nearly the size of a human

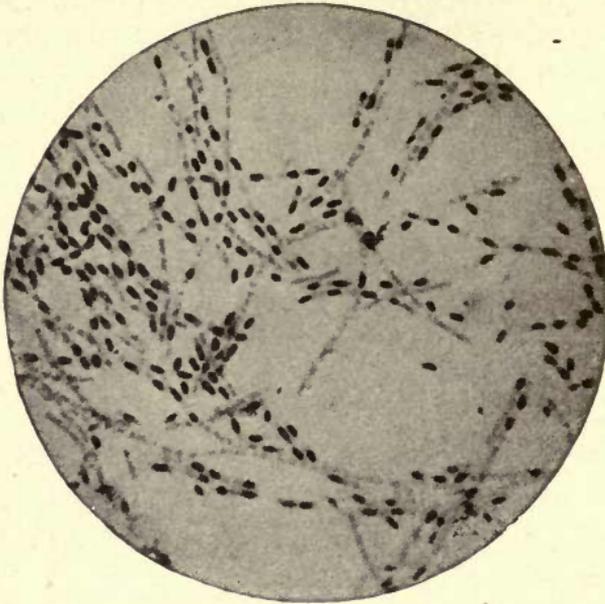


Fig. 53.—*Bacillus anthracis*, stained to show the spores ($\times 1000$) (Frankel and Pfeiffer).

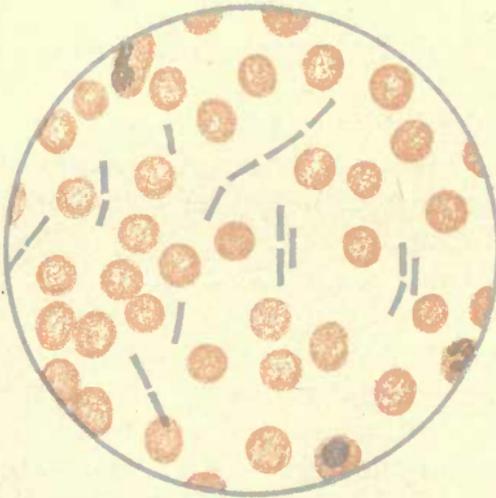


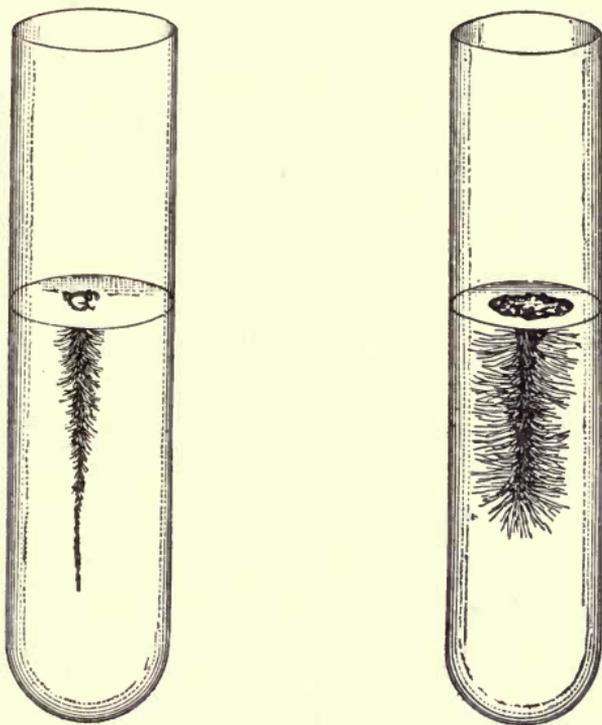
Fig. 54.—Anthrax bacilli in human blood (fuchsin staining) (Zeiss one-twelfth oil-immersion; No. 4 ocular) (taken from Vierordt).

blood-corpuscle; broad, cup-shaped ends; in bouillon cultures long threads are formed, with *large oval spores* (Figs. 53, 54).

Properties.—Liquefies gelatin; immotile; the spores are very resisting, living twenty years, and resist boiling for five minutes.

Growth.—Grows rapidly, between 12° C. and 45° C., and requires plenty of oxygen, but may be classed as a facultative anaërobe; grows well in all media.

Plates of Gelatin.—Colonies develop in two days; white shiny spots, which appear under microscope as slightly yellowish



Figs. 55, 56.—Stab-cultures of anthrax in gelatin.

granular twisted balls, like a ball of yarn; each separate string or hair, if looked at under high power, being composed of bacteria in threads.

Stab-cultures.—A white growth with thorn-like processes along the needle-track; later on, gelatin liquefied, and flaky masses at the bottom. (See Figs. 55, 56.)

Potato.—A dry, creamy layer, and when placed in brood-oven, rich in spores.

Varieties.—*Asporogenic.*—By cultivation in gelatin, containing 1 : 1000 phenol, a variety develops that cannot produce spores. Also *involution forms*, differing from the usual type.

Staining.—They readily take all the anilin dyes with the ordinary methods. To bring out the cup-shaped concave extremities, a very weak watery solution of methylene-blue is best.

Spores are stained by the usual method. When several bacilli are joined together, the place of their joining looks like a spore, because of the hollowed ends. The double staining will develop the difference.

Sections of tissue are stained according to the ordinary methods, taking *Gram's* method very nicely.

Pathogenesis.—When mice are inoculated with anthrax material through a wound in the skin, they die in twenty-four hours from an active septicemia, the point of inoculation remaining unchanged. The following appearances then present themselves:

Peritoneum.—Covered with a gelatinous exudate.

Spleen.—Very much swollen, dark red, and friable.

Liver.—Parenchymatous degeneration.

Blood.—Dark red. The bacilli are found wherever the capillaries are spread out, in the spleen, liver, intestinal villi, and glomeruli of kidney, and in the blood itself. Only when the capillaries burst are they found in the tubules of the kidney.

Mode of Entrance.—The bacilli can be *inhaled*, and then a pneumonia is caused, the pulmonary cells containing the bacilli; when the spores are inhaled, a general infection occurs.

Feeding.—The cattle graze upon the meadows, where the blood of anthrax animals has flowed and become dried, the spores remaining, which then mix with the grass and so enter the alimentary tract; here they then cause the intestinal form of the disease, ulcerating through the villi.

Local Infection.—In man usually only a local action occurs; by reason of his occupation—wool-sorter, cattle-driver, etc.—he obtains a small wound on the hand, and local gangrene and necrosis set in, but death follows in the severer forms from a

general pyemia; there is severe edema of the tissues in and about the wound, and a pulmonary edema likewise occurs. Wounds about the face and neck are more fatal.

Pneumonia by inhalation and intestinal infection also occurs in man.

Susceptibility of Animals.—Dogs, birds, and cold-blooded animals affected the least; while mice, sheep, and guinea-pigs quickly and surely.

Products of Anthrax Bacilli.—A basic ptomain has not been found, but a toxalbumin or proteid, called *anthraxin*, has been obtained. A certain amount of *acid* is produced by the virulent form, *alkali* by the weak.

Attenuation and Immunity.—Cultures left several days at a temperature between 40° and 42° C. soon become innocuous, and when injected into animals protect them against the virulent form.

The lymph obtained from lymph-sac of a frog destroys the virulence of anthrax bacilli and spores temporarily.

Hankin obtained an alexin from the blood and spleen of rats, they being naturally immune. It destroyed the anthrax bacilli in vitro, and used by injection in susceptible animals, made them immune. It is insoluble in alcohol or water.

Protective Vaccination.—Animals have been rendered immune by various ways—by inoculation of successive attenuated cultures; also with sterilized cultures—that is, cultures containing no bacilli, and with cultures of other bacteria.

Habitat.—In the serum about the wound and in the blood anthrax bacilli are readily found. Tannery employes are frequent sufferers.

The bacillus has never been found free in nature.

Bacillus Tuberculosis (Koch).—This very important bacillus was first described, demonstrated, and cultivated by Koch, who made his investigations public on the 24th of March, before the Physiological Society of Berlin, in the year 1882.

Origin.—In various tuberculous products of man and other animals.

Form.—Very slender rods, nearly straight, about one-quarter

the size of a red corpuscle's diameter, their ends rounded, usually solitary, often, however, lying in pairs in such a manner as to form an acute angle. Sometimes they are S-shaped. In colored preparations little oval spaces are seen in the rod, which resemble spores; but the question of the existence of spores is still undecided. (See Figs. 57, 58.)

Properties.—Does not possess self-movement.



Fig. 57.—Tubercle bacilli in sputum; carbol-fuchsin and methylene-blue (Zeiss one-twelfth oil-immersion).

Growth.—Requires special media for its growth, and a temperature varying but slightly from 37.5°C . It grows slowly, developing first after ten days, reaching its maximum in three weeks. It is facultative anaërobic. On gelatin it does not form a growth.

Colonies on Blood-serum.—Koch first used blood-serum for culture, and obtained thereon very good growths. Test-tubes with *stroke* culture are placed in the brood-oven at 37°C . for ten to fourteen days, when small glistening white points appear, which then coalesce to form a dry, white, scale-like

growth. Under microscope composed of many fine lines containing the tubercle bacillus.

Glycerin-agar.—By adding 4 to 6 per cent. glycerin to ordinary agar-peptone medium, Nocard and Roux obtained a culture-media upon which tubercle bacilli grow much better than upon blood-serum. This is now almost exclusively used.

Stroke cultures are here used as with blood-serum. They are placed in incubator after inoculation, and remain there about ten days, at a temperature of 37° C. The cotton plugs of the tubes are covered with rubber caps, the cotton first having been



Fig. 58.—Giant-cell containing bacilli (from a photograph made by Dr. Wm. M. Gray).

passed through the flame, and moistened with a few drops of sublimate solution. The rubber cap prevents the evaporation of the water of condensation, which always forms and keeps the culture from drying up.

The growth which occurs resembles the rugæ of the stomach, and sometimes looks like moistened crumbs of bread. The impression or “Klatsch” preparation shows under the microscope a thick, curled-up center around which threads are wound in all directions. And these fine lines show the bacilli in profusion.

Potato.—It can be cultivated on slices of potato which are placed in air-tight test-tubes.

Bouillon.—Bouillon containing 4 per cent. glycerin is a very good nurture ground.

Varieties.—Branching and other aberrant forms are not rare, and the tendency now is to class the organism with the "higher bacteria." Other acid-fast bacilli exhibit similar types, and it is possible that the bacillary parasitic form is only one stage in the life-history of the organism.

Little granules, arranged like streptococci, which take the characteristic stain, and look as if the protoplasm had been

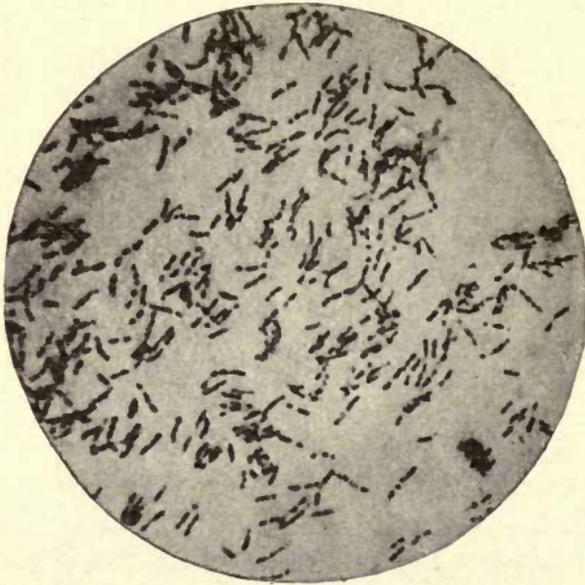


Fig. 59.—Tubercle bacillus in sputum (Fränkel and Pfeiffer).

destroyed that inclosed them, are frequently found in sputum.

Bovine tubercle bacilli are about one-third smaller than human tubercle bacilli.

Staining.—The tubercle bacilli require special methods to stain them, and a great number have been introduced. They are stained with great difficulty: but once stained, they are very resistant to decolorizing agents. Upon these facts all the methods are founded.

It will be necessary to describe only those methods principally in use; and as the examination of sputum for bacilli is of so

frequent an occurrence, and so necessary, it is well to detail in particular the method of staining.

Starting with the sputum, we search for little clumps or rolled-up masses; if these are not present, the most solid portions of the mucus are brought with forceps upon a clean cover-glass; very little suffices. With another cover-glass it is pressed and spread out evenly; drawing one glass over the other, we obtain two specimens, and these are put aside or held high over the flame until dry.

If we desire to examine the specimen quickly, or make a hurried diagnosis, we use the *rapid* method, with hot solutions; otherwise we let it stay in cold solution until the next morning, the advantages of which will be later on described.

The Rapid Method (B. Fränkel's Method, Modified by Gabbet).—The principle is to combine with the contrast stain the decolorizing agent; but the preparations are not permanent; the method, however, is very useful.

Two solutions are required: one of Ziehl's carbol-fuchsin; the other Gabbet's acid methylene-blue. (See Formula No. x, on p. 33.)

The cover-glass containing the dried sputum is passed three times through the flame, as described in the general directions. It is then placed in the carbol-fuchsin solution five minutes (cold), or two minutes in the hot, immediately then transferred to the second solution, the acid blue, where it remains one minute, then washing in water. The preparation is dried between filter-paper, and mounted best first in water. Examined with oil-immersion.

A somewhat longer, but preferable, method is to decolorize the carbol-fuchsin with weaker acid. The smear is treated with

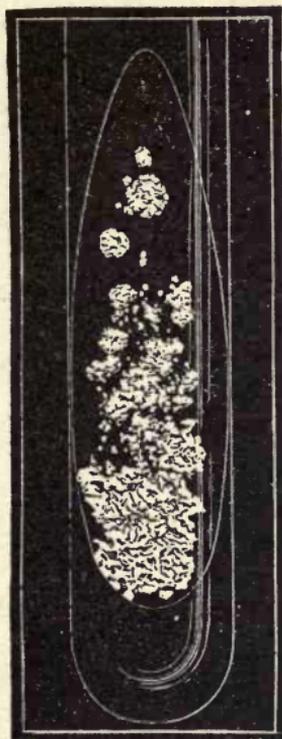


Fig. 60.—Growth on agar.

5 per cent. nitric or 10 per cent. sulphuric acid until, after washing with water, a bright pink remains. The excess of color is then washed out with 95 per cent. alcohol until no further color is imparted to the alcohol, and the smear is a pinkish gray. The preparation is then washed with water and counterstained with aqueous methylene-blue for ten to thirty seconds. A mechanical stage is of great assistance in the search for the bacilli, as it permits every portion of the preparation to be inspected systematically.

Slow Method.—The stain may also be used without heating, though in this case a much longer time is required before the bacilli take up the stain. The preparation is left in a small dish or beaker full of carbol-fuchsin for eight to ten hours, and then decolorized and counterstained in the usual way. The method is less liable to produce artefacts than the quick method, but is not much used on account of the time it takes.

Examination in Urine.—In urine, owing to the almost inevitable contamination with the smegma bacillus, special methods are necessary to avoid error. The preparation may be left in 97 per cent. alcohol for eight hours, when the smegma bacillus will have become decolorized, or Pappenheim's method may be used: (1) Smear and fix as usual; (2) stain with hot carbol-fuchsin for two minutes, pour off the surplus dye without washing; (3) counterstain and decolorize by pouring five times over the preparation the following solution: A 1 per cent. alcoholic solution of corallin is saturated with methylene-blue and 20 parts of glycerin added. Wash in water, dry with blotting-paper, then in the air, and examine. The tubercle bacilli are stained red, smegma bacilli, blue.

Examination of Milk for Tubercle Bacilli.—Place a drop of the sample on a cover-glass and mix it with two drops of a 1 per cent. solution of sodium carbonate. The cover-glass is then gently warmed until evaporation is complete. The saponified fat is then stained, as the ordinary cover-glass preparation. Only a very few persons have succeeded in discovering the bacillus in milk.

The bacillus of leprosy resembles the tubercle bacillus in its staining properties, but gives up the carbol-fuchsin more easily and is usually decolorized by the acid and alcohol. It is colored blue by Pappenheim's method.

Acid-fast bacilli have also been obtained from timothy grass, butter, milk, manure, and the surfaces of animal bodies, but differ from the tubercle bacillus in cultural characteristics.

Biedert's Method of Collecting Bacilli.—When the bacilli are very few in a great quantity of fluid, as urine, pus, abundant mucus, etc., Biedert advises to mix 15 c.c. of the fluid with 75 to 100 c.c. water and a few drops of potassium or sodium hydroxid, then boiling until the solution is quite thin. It is placed in a conical glass for two days, and bacilli with other morphologic elements sink to the bottom of the glass; when the supernatant liquid is decanted, the residue can be easily examined. In this way bacilli were found that had eluded detection examined in the ordinary manner.

The centrifugal machine is used either in connection with Biedert's sediment method or without, to obtain the solids suspended in urine or serum.

When the bacilli are so few in number in sputum or urine as to make their detection difficult, and also when doubt exists as to the identity of acid-fast bacilli found, several guinea-pigs should be injected in the groin and smears and sections made from the enlarged glands resulting.

Phenol to Sediment Sputum.—Pure phenol added to sputum (about 1 part of the acid to 6 parts of sputum) will in a few hours produce a coagulation and allow the sputum to be spread evenly on the cover-glass, showing greater collections of bacilli.

Without Cover-glass.—Sputum can be spread and stained on the glass slide without the use of a cover-glass, the oil of cedar being placed directly on the stained sputum, and the oil-immersion lens dipping into it. It is a rapid and cheap way; and when a given case is to be studied daily, the method is useful.

Pure Cultures from Sputum.—Kitasato recommends the thorough washing, changing the water ten times, of the small masses found in the sputum of tuberculous persons. When

such specimens are examined, they show tubercle bacilli alone, and when inoculated in agar, give rise to pure cultures.

Staining Bacillus Tuberculosis in Tissue (Sections).—The general method of Gram can be used, but the better way is to use the following:

- Carbol-fuchsin, fifteen to thirty minutes.
- 5 per cent. sulphuric acid, one minute.
- Alcohol, until a light-red tinge appears.
- Weak methylene-blue, three to five minutes.
- Alcohol, for a few seconds.
- Oil of cloves, until cleared.
- Canada balsam, to mount in.

Instead of carbol-fuchsin, *alcoholic solution of fuchsin* or *anilin-water fuchsin* can be used, but the sections must remain in the stain overnight.

Hardened Sputum and Sectioning.—Sputum can be hardened by placing it in 98 per cent. alcohol. Thin sections can be obtained by imbedding the hardened sputum in collodion. The sections are then stained as ordinary tissue sections.

To Preserve Sputum.—Sputum can be preserved for future use by placing it in alcohol, where it can be kept for months. Cover-glass preparations can then be made by softening the coagula with a small amount of liquor potassa.

The resisting action of the bacillus to acids is supposed to be due to a peculiar arrangement of the albumin and cellulose of the cell, rather than to any particular capsule around it.

Pathogenesis.—When a guinea-pig has injected into its peritoneal cavity some of the diluted sputum containing tubercle bacilli, it perishes in about three weeks, and the following picture presents itself at the autopsy: at the point of inoculation there is a local tuberculosis, little tuberculous nodules containing the characteristic bacilli. In the lungs and the lymphatics, similar tubercles are found—a general tuberculosis.

If the animal lingers a few weeks longer, the tubercles becomes necrosed in the center and degeneration occurs, the

periphery still containing active bacilli, cavities having formed in the center.

Since the bacilli die in course of time, killed by their own products, their number forms no correct guide of the damage present: even their absence in the sputum does not preclude the absence of a tuberculous process. *It is their presence only that warrants a positive declaration.* The number of bacilli in a given specimen is no indication of the severity of the disease.

They are found in the blood only when a vessel has come in direct contact with a tuberculous process through rupture or otherwise. They have been found in other secretions—milk, urine, etc.

Man is infected as follows:

Through Wounds.—Local tuberculosis.

Through Nutrition.—Milk and meat of tuberculous animals. Phthisical patients swallowing their own sputum and causing an intestinal tuberculosis.

Inhalation.—This is the most usual way, probably constituting the cause in nine-tenths of the cases, except in children.

The sputum of phthisical patients expectorated on the floors of dwelling-houses, in handkerchiefs, etc., dries, and the bacilli set free are placed in motion by the wind, or rising with the dust, are thus inhaled by those present. When the sputum is kept from drying by expectoration in vessels containing water, this *great danger* can be *avoided*.

Nearly all the cases of *heredity* can be explained in this manner; the young children, possessing very little resistance, are constantly exposed to the infection through *inhalation*, and are especially prone to intestinal infection through milk and other foods.

Immunity.—No one can be said to be immune, though persons who have been greatly weakened offer less resistance than healthy individuals.

Tuberculosis in Animals.—Tuberculosis is probably the most widely disseminated disease among domestic animals, and affects cattle, pigs, horses, dogs, cats, the smaller ruminants, birds, and even turtles and fish. The conclusion of Koch,

made public in his address to the Tuberculosis Congress in 1901, that human and bovine tuberculosis are distinct and that infection of human beings from cattle occurs so seldom that no general regulations to restrict it are necessary, has found few adherents. It is true that certain differences exist between human and bovine tubercle bacilli, the latter appearing to be more virulent to animals, and it is a fact that cattle are very slightly susceptible to the human bacillus, but it is not likely that the converse is so. Children are particularly liable to infection through the gastro-intestinal tract, and it has been shown that the uninjured mucosa of the infant's intestine is permeable to bacilli, so that the pulmonary disease in the young may often be the result of tuberculous bronchial nodes secondary to tuberculous glands of the mesentery.

Various observations on animals have shown that the bacillus occurring in each species has acquired certain special characteristics regarding growth and virulence. The bacilli causing tuberculosis in the cold-blooded animals have departed farthest from the human type, those of birds to a less degree, and those of cattle least of all.

Products of Tubercle Bacilli.—The true nature of the tubercle toxin is not yet clear. It is not unlikely that several toxic bodies differing from one another in their properties are produced. Koch's tuberculin (1890) was obtained by filtering, through unglazed porcelain, concentrated glycerin bouillon cultures of tubercle bacilli. It was speedily shown to be devoid of curative power, and is now used mainly for diagnosing the disease in cattle. In healthy animals little or no reaction is produced by the injection of 30 to 40 cg. of tuberculin, but if tuberculous, the temperature rises 2° or 3° F. in eight to twelve hours, and remains elevated for a like period of time.

Tuberculocidin.—This is an albuminoid obtained from the original tuberculin by precipitation with alcohol. Klebs used it as a cure for tuberculosis.

Tuberculin R is an extract made from dried and powdered living bacilli, and was recommended by Koch in place of the original tuberculin.

Bacillen emulsion (B. E.) is similar to tuberculin R, and is a glycerin emulsion of crushed bacteria. Theo. Smith recommends virulent uncrushed bacteria killed by moderate heat.

Denys' B. F. tuberculin is a filtrate of liquid cultures to which 0.25 per cent. phenol has been added and allowed to stand two weeks. It is prepared in eight dilutions.

Opsonic Treatment.—In recent years the use of tuberculin R has again been brought forward by Wright and others and curative claims made for it. It is used in very small doses, $\frac{1}{10000}$ milligram at intervals of several days, and the effect on the opsonic index carefully watched.

Use of Tuberculin.—In the use of tuberculin severe reactions are to be avoided. The smallest dose possible is commenced with. *Trudeau* uses for afebrile cases $\frac{1}{10,000}$ milligram liquid measure Koch's B. E., or Denys' B. F., increasing 1 decigram every three days until 1 c.c. can be injected without causing any reaction. This treatment must extend over months. Tuberculin immunity does not last indefinitely.

Ophthalmic Tuberculin Reaction of Calmette.—A modified form of tuberculin is placed on the conjunctiva of an individual suspected of having tuberculosis. In a few hours a congestion, more or less severe, results, and lasts several days. In healthy persons no reaction occurs. The test is claimed to be harmless, though severe reactions have been reported, in tuberculous patients, and even in healthy persons a second application to the same eye may cause an inflammatory reaction.

Agglutination.—*Arloing* and *Courmont* have described an agglutination reaction for the tubercle bacillus similar to the *Widal* reaction of typhoid fever (see page 131). It is very unreliable, however, and but little importance is attached to it.

Antituberculous Serum.—The attempts to produce an effective serum have so far been unsuccessful. *Marmorek*, by growing the bacillus on a special serum obtained by injecting calves with the leukocytes of guinea-pigs, has secured a toxin which he used to immunize horses, and the serum so obtained has been tried with encouraging results, but its value is still

doubtful. Several other serums have been introduced, but none have shown any lasting virtues.

Lepra Bacillus (Hansen).—*Origin.*—In 1880 Armauer Hansen declared, as the result of many years' investigation, that he found a bacillus in all leprous processes.

Form.—Small slender rods, somewhat shorter than tubercle bacilli, otherwise very similar in appearance.

In the interior of the cell two or three oval spaces are usually seen, not known if spores or otherwise.

Properties.—They are immotile, and do not liquefy the nutrient media.

Growth.—Bordoni-Uffreduzzi have obtained growths upon blood-serum to which peptone and glycerin had been added, but the accuracy of this observation is very doubtful.

Staining.—They resist the decolorizing action of acids, as the tubercle bacilli, but they are easily stained, requiring but a few minutes with the ordinary watery solutions. They take Gram's stain readily.

Pathogenesis.—Arning has inoculated prisoners with tissue obtained from leprous patients, and produced true leprosy.

Rabbits which had been infected through the anterior chamber of the eye showed the lepra nodules (containing the lepra bacilli) diffused through various organs, but here again the results are not wholly satisfactory.

In man the skin and peripheral nerves are principally affected, but the lymphatic glands, liver, and spleen can also become the seat of the lepra nodules. The lepra cells which compose these nodules contain the bacilli in large numbers. By applying a vesicant to the leprous skin, the serum thereby obtained will contain great numbers of bacilli. This is a simple diagnostic test.

Method of Infection.—Not yet determined; the air, soil, water, and food of leprous districts have been carefully examined without result. The nasal secretion is very infectious.

Syphilis Bacillus of Lustgarten (Smegma Bacillus of Alvarey and Tavel).—Lustgarten, in 1885, through a certain staining process, found peculiar bacilli in syphilitic tissues

which he thought had a direct connection with the disease. But this has been disproved and the cause has been found in a protozoön which has been called *Spirochæta pallida*, which see (p. 194).

Bacillus of Glanders (*Bacillus Mallei* (Löffler-Shütz); *Rotz-bacillus*).—*Origin*.—In the “farcy buds” or little nodules of the disease, by Löffler and Shütz, in 1882.

Form.—Small slender rods, about the size of the tubercle bacillus. The ends rounded. Never appearing in large collec-



Fig. 61.—Bacillus of glanders from a culture upon glycerin agar-agar ($\times 1000$) (Fränkel and Pfeiffer).

tions, usually singly. *Spores* are said to exist, but this is doubtful (Fig. 61).

Properties.—The rods are very resistant, living in a dried state for three months and longer without any spores present. They are not motile; possess, however, great molecular vibration.

Growth.—The growth occurs between 25° and 40° C.—best at 37° C.; it is very sparse upon gelatin, but on glycerin-agar or blood-serum a very abundant growth occurs.

Colonies.—On agar or glycerin-agar there appear in two to three days small white glistening drops, which under microscope seem as round granular masses with an even periphery.

Stroke Cultures.—On glycerin-agar and blood-serum small transparent drops of whitish or grayish color, which soon coalesce to form a broad band.

Potato.—An amber-colored, honey-like growth which gradually turns red, then brown, and greenish-brown around it. Weakly acid potatoes are a good medium and give the most typical growth.

Staining.—Since the bacillus is very easily decolorized, some special methods have been recommended.

Löffler's (for cover-glass preparations):

1. Alkaline methylene-blue (Löffler's), five minutes.
2. Acetic acid with a few drops of tropæolin, one second.
3. Washed in water.

For Sections.—Instead of tropæolin acetic acid, the following mixture is used:

Oxalic acid (5 per cent.).....	1 drop.
Concentrated sulphuric acid.....	2 drops.
Distilled water.....	2 drams.—M.

The sections are kept in this five seconds.

Kühne's method (cover-glass):

1. Warm carbol-blue, two minutes.
2. Decolorized in weak solution of muriatic acid (10 : 500).
3. Washed in water.

Sections of tissue:

1. Carbol-blue, one-half hour.
2. Decolorized in $\frac{1}{2}$ per cent. muriatic acid.
3. Washed in distilled water.
4. Dehydrated in alcohol one second.
5. Anilin-oil with 6 drops of turpentine, five minutes.
6. Turpentine, xylol, Canada balsam.

If contrast stain, add 5 drops of safranin (Bismarck-brown) to turpentine, and use it after the xylol.

Pathogenesis.—If horses, field-mice, or guinea-pigs be inoculated subcutaneously, with but a very small quantity of culture, a local affection results, followed some time after by a general disturbance; ulcers form at the point of inoculation; little nodules, which then caseate, leaving scars and involving the lymphatics; metastatic abscesses then occur in the spleen and lungs, and death arises *from exhaustion*. Cattle, pigs, and rabbits are not easily affected; man is readily attacked. The bacilli gain entrance to the blood and urine. Nasal glanders occurs whatever the mode of inoculation.

Manner of Infection.—Glanders being a highly contagious disease, it requires but a slight wound to allow it to gain entrance.

In horses the primary sore seems to be at the nasal mucous membrane. In man it is usually on the fingers. Boiling water or 1 : 10,000 sublimate solution will quickly destroy the virulence of this bacillus.

Mallein.—A substance called *mallein* has been obtained from the cultures grown in glycerin bouillon. It gives a reaction when injected into cattle suffering from glanders, and is said to be useful in diagnosing the disease.

Bacillus of Diphtheria (Klebs-Löffler).—*Origin.*—Klebs found it in membrane in 1883; it was isolated by Löffler in 1884.

Form.—Small, slightly curved rods about as long as tubercle bacilli and twice as broad; the ends are at times swollen; spores have not been found. Their form is, however, very variable—sometimes much longer than usual, one end often greatly knobbed. Normal bacilli are found only in membrane.

Stained forms are characteristic, since the ends are more easily colored than the center, and usually the bacillus stains in segments, so that it seems to be made up of very short sections. At first sight it appears like a chain of cocci.

Small granules, the Babes-Ernst granules, are shown by the special staining of Neisser.

Properties.—They do not possess any movement; do not

liquefy gelatin. They are not very resistant, being destroyed by a temperature of 50° C., but they have lived on blood-serum five months. Acid is produced in sugar media.

Growth.—Grow readily on all media, but best on blood-serum mixtures, between temperatures of 20° and 40° C. They are facultative anaërobic; they grow quite rapidly and profusely. Egg cultures (Hueppe's method) give good growths. Passing currents of air increase the growth.

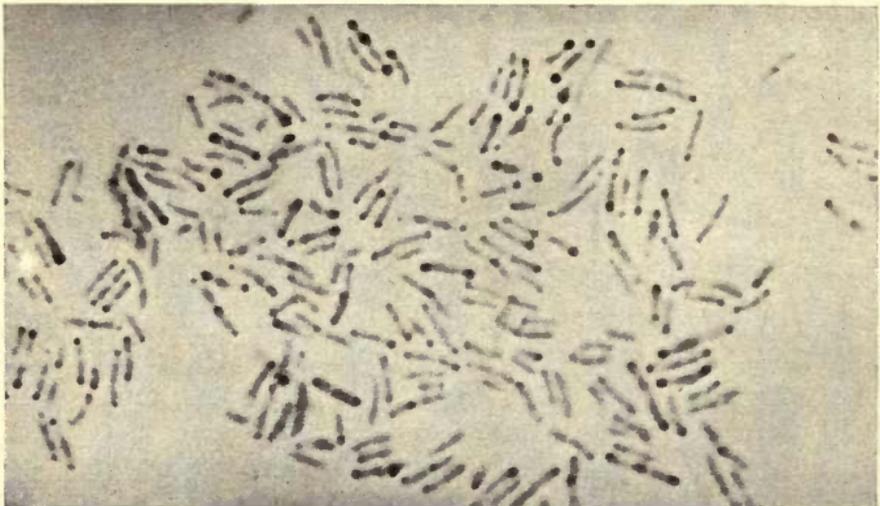


Fig. 62.—Diphtheria bacilli from a culture on blood-serum, stained by Löffler's methylene-blue solution, showing deeply stained points; ($\times 2000$) (Wright and Brown).

Colonies on Gelatin Plates.—At 24° C. little round colonies, white under low power, granular center; irregular borders.

Stab-cultures.—Small, white drops along the needle-track. In glycerin-agar a somewhat profuse growth.

Potato.—On alkaline surface, a grayish layer in forty-eight hours.

Blood-serum (After Löffler).—Blood-serum 3 parts, and bouillon 1 part; the bouillon contains peptone, 1 per cent., sodium chlorid, $\frac{1}{2}$ per cent., and dextrin (or glucose), 1 per cent.

In a few hours (eight to sixteen) on the white opaque surface a slight moisture is noticeable which, if examined, is composed

of bacilli. In twenty-four hours small round colonies are found which seem to arrange themselves concentrically. The growth becomes more abundant, and the individual colonies larger and yellowish. On *blood-coagulum* the growth is usually gray and the margins of the culture crenated. Often a diagnosis can be made in four hours if the serum-tubes are kept in a brood-oven.

Serum-agar.—Joos finds *serum-agar* better than Löffler's serum: 300 c.c. blood-serum mixed with 50 c.c. normal soda solution and 150 c.c. water, heated in water-bath for two to three hours at 60° to 70° C., then raised to 100° C., or in steam-chest one-half hour. Then 500 c.c. peptone bouillon (slightly alkaline) and 20 gm. agar. When the agar is dissolved by heat, avoiding prolonged boiling, the mixture is filtered and sterilized one-quarter hour at 100° to 110° C. in autoclave; then poured into Petri dishes. Streptococci do not grow on this medium, whereas diphtheria bacilli will grow in from six to twelve hours.

Bouillon.—In bouillon an abundant growth takes place, and this medium is used to obtain the *toxins*.

Staining.—Is positive by Gram's method. Stained best with Löffler's alkaline methylene-blue. Neisser's double stain (see p. 34) shows granules, blue black, and body, brown.

Pathogenesis.—By inoculation, animals, which naturally are not subject to diphtheria, have had diphtheritic processes develop at the site of infection; hemorrhagic edema then follows, and death.

No agglutinins are developed in the serum.



Fig. 63.—*Bacillus diphtheriae*; agar-agar culture (photograph by Dr. Henry Koplik).

In rabbits paralysis develops, and when the inoculation occurs upon the trachea, all the prominent symptoms of diphtheria show themselves.

Manner of Infection in Man.—The exact way is not yet known. It is supposed that the mucous membrane, altered in some manner, the diphtheria bacillus then gains entrance and the disease develops. The bacilli may be found in healthy individuals who may act as a source of infection to susceptible individuals without themselves becoming infected.

Prevalence of Bacillus Diphtheriæ.—Examinations made on a large scale of the throats of supposedly healthy individuals



Fig. 64.—*Bacillus diphtheriæ*, from a pure culture.

have shown that the *Bacillus diphtheriæ* is rather widely distributed. Not only does it linger for many weeks in the throats of persons recently recovered from the disease, but it is found in the care-takers, nurses, etc., and there are allied organisms, with more or less pathogenicity, that have been found in atrophic rhinitis, in conjunctivitis, and in the throats of unexposed normal individuals. The *pseudobacillus* of Hoffman is believed by many investigators to be but a weakened diphtheria bacillus that has lost its toxic power.

Methods of Diagnosis.—A small piece of exudate or some secretion from pharynx, tonsil, or nares is obtained on a sterile cotton swab and transferred, as soon as possible, to the surface

of two or more blood-serum tubes (if these are not available, the swab should be placed in a sterile test-tube or bottle, and sent to the laboratory at once). The inoculated tubes are placed in the incubator at 37° C., and examined in twelve hours. If a growth is visible, a slide is made and stained with Löffler's and Neisser's stain, and if bacilli are present, with characteristic granules, the diagnosis of diphtheria is most probable. If there are no clinical signs, the growth should be

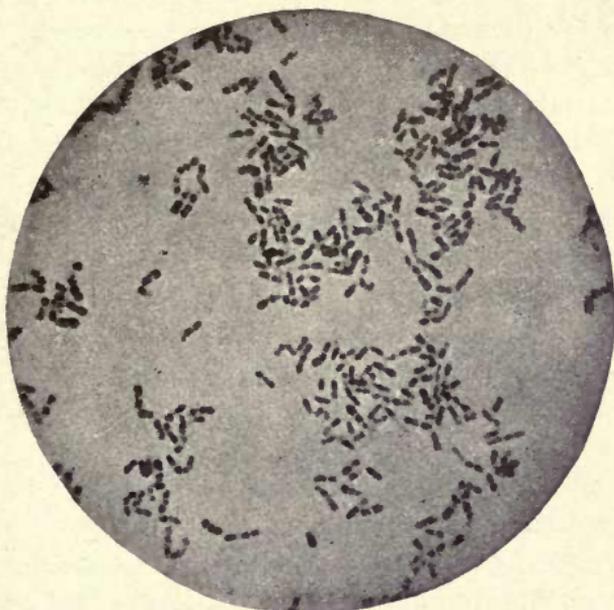


Fig. 65.—*Bacillus diphtheriæ*, from a culture upon blood-serum ($\times 1000$) (Fränkel and Pfeiffer).

tested for toxicity by inoculating a guinea-pig; it should be grown in alkaline sugar bouillon and tested in two days for acid. The xerosis and Hoffman's bacilli are not pathogenic for guinea-pigs and do not produce an acid reaction in sugar media.

Products.—But it is not the mere presence of the bacillus that gives rise to all trouble; certain products which they generate get into the system and produce the severe constitutional symptoms.

Roux and Yersin, in 1888, discovered that the injection of the filtered culture bouillon (that is, freed of all diphtheria bacilli) gave rise to the same palsies as when the bacilli themselves were introduced.

Toxins of Diphtheria.—The toxins may be separated from three-weeks-old bouillon cultures by filtration. They are not albumins and are very complex. Ehrlich claims two principles: one he calls *toxone*. The other, *toxin*, the toxone produces paralytic symptoms, and appears to be less affected by antitoxin. The toxins are highly poisonous—0.001 c.c. may be sufficient to kill a guinea-pig in less than twenty-four hours. The substance is unstable, losing its toxic power gradually. Heating at 58° C. for two hours is destructive, but drying renders it more stable.

Antitoxin.—Behring found that animals rendered immune had a principle in their blood that was antagonistic to the development of the toxin.

Immunity.—Brieger and Fränkel, by injecting 10 to 20 c.c. of a three-weeks-old culture of diphtheria bacilli, which had been heated at 70° C. for one hour, produced an immunity in guinea-pigs against the virulent form.

The strength commonly employed in human beings is 3000 units, and as much as 20,000 units may be given without detriment in severe cases. If this amount is injected into a child suffering from diphtheria in the earlier stages (second to third day), the disease is often arrested. The membrane begins to disappear, and in two or three days has vanished. The constitutional symptoms are likewise greatly influenced by the injection. For prophylaxis and immunizing well persons 1000 to 1500 units are employed.

In such conditions as asthma severe and fatal results have followed the use of the serum.

The *antitoxin* has no influence on the bacteria themselves; their virulence and length of residence in the body are not lessened.

The toxin generated by the germ is supposed to be neutralized by the antitoxin and prevented from injuring the body tissues.

Preparation of Toxin.—The bacillus is grown in muscle-sugar-free bouillon with an alkaline reaction. Acids prevent toxin formation. There should be a free supply of oxygen, and, therefore, large shallow flasks are used. The maximum toxicity is developed in seven to ten days. The strength should be $\frac{1}{500}$ c.c., fatal for 500-gram guinea-pig.

Preparation of Antitoxic Serum.—Horses are rendered immune by gradually increased doses of diphtheria toxin, the power of the toxin having first been standardized by its neutralization with some standard antitoxin in powdered form. The toxin is at first injected subcutaneously, then intravenously, and after several months' treatment a resistance is obtained that will withstand 300 to 500 times the original lethal dose. The horse is then bled, and from five to nine liters withdrawn, this is then allowed to coagulate, and under very careful precautions the serum is placed in sterile packages, its strength having first been compared with a standard furnished by the United States Government.

Antitoxic Unit.—A normal antitoxic serum is one that contains in each cubic centimeter one immunity unit. An *immunity unit*, according to Ehrlich, is the amount of antitoxic serum which will neutralize 100 times the minimum lethal dose of toxin, when serum and toxin mixed and injected into a 250-gram guinea-pig does not cause death in four days. Thus, if the serum will protect in doses of $\frac{1}{50}$ c.c., then each cubic centimeter has 50 units power, and 20 c.c. will contain 1000 units, or will be sufficient to neutralize an amount of toxin that would be fatal for 25,000 kilos (12,500 pounds) of guinea-pigs, or 100,000 pigs weighing 250 grams each.

Streptococcus in Diphtheria.—Streptococci have been found quite constant in diphtheria, but they resemble the *Streptococcus pyogenes*, and have no specific action.

Bacillus of Typhoid or Enteric Fever (Eberth-Gaffky).—*Origin.*—Eberth found this bacillus in the spleen and lymphatic glands in the year 1880, and Gaffky isolated and cultivated the same four years later.

Form.—Rods with rounded ends about three times as long as

they are broad. Usually solitary in tissue-sections, but in artificial cultures found in long threads. Flagella on the side.

Properties.—They are very motile; they take the anilin dyes less deeply than some similar bacilli. *Spores* have not yet been found; they do not liquefy gelatin.

Growth.—They are facultative anaërobic; grow best at 37° C., but can also develop at ordinary room temperature. All nutrient media can be used as culture-ground. They develop chiefly on the surface, and very slowly. Repeated freezing and



Fig. 66.—*Bacillus typhi*, from an agar-agar culture six hours old, showing the flagella stained by Löffler's method ($\times 1000$) (Fränkel and Pfeiffer).

thawing do not affect the vitality of the germ, and phenol in 1 to 2 per cent. solution has no effect on it. A ten-minute exposure to 60° C. is invariably fatal.

Colonies on Gelatin Plates.—Two forms; the ones near the surface spread out like a leaf, transparent, with bluish fluorescence. The deeper ones resemble whetstone crystals of uric acid, with the same yellowish tinge.

In five days they attain to 3 millimeters in diameter.

On Potato Gelatin.—The colonies do not have the yellow

color, they are transparent; later on they become dark brown with green iridescence.

Stab-cultures.—Mainly on the surface, a pearly layer.

Stroke Cultures.—A transparent thick layer.

Potato.—The growth here is quite characteristic. At 37° C. in forty-eight hours a moist, transparent film is formed over the whole surface, but so transparent that it can hardly be seen without close observation. If a small portion of this is placed under a microscope, it will be seen swarming with bacilli.

The growth never becomes more prominent; the potato must have a neutral or acid reaction.



Fig. 67.—Typhoid fever bacillus in pure culture ($\times 650$).

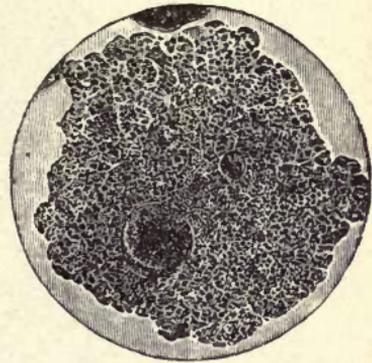


Fig. 68.—Colonies of typhoid bacilli three days old ($\times 100$) (Fränkel and Pfeiffer).

Milk.—The bacteria grow very well in milk, producing a slightly acid reaction, but no *coagulation*.

Phenol Gelatin.—Gelatin which has added to it $\frac{1}{10}$ per cent. phenol will allow the typhoid bacillus to develop, other similar bacilli being destroyed.

Glucose Gelatin.—In glucose gelatin there is no gas-production. Indol is likewise not generated by the typhoid bacillus, whereas it is by the colon bacillus. On *Elsner's potato-gelatin* the colon bacillus and the typhoid bacillus grow readily. The *medium of Hiss* is of great assistance in isolating the germ.

The *Gruber-Widal blood-serum test*, or, as it is otherwise known, the agglutination phenomenon (Fig. 69), has the following history:

About 1889, Charrin and Roger observed in the serum of immunized animals that the *Bacillus pyocyaneus* arranged itself in little clumps. Other investigators reported the same thing for other bacteria, and Metchnikoff added that motility was destroyed.

In 1895 Bordet showed that the serum of cholera-immunized animals, when mixed with bouillon cultures of cholera spirilla, affected their motility and caused them to form masses, or "Klumpen," as the Germans call it.

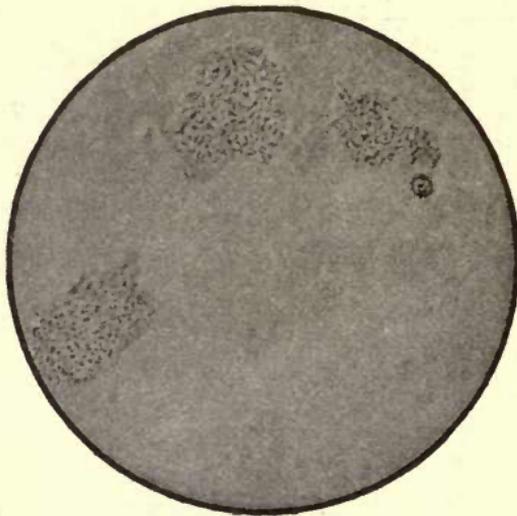


Fig. 69.—The Widal agglutination reaction (Slater and Spitta).

R. Pfeiffer, in the same year, showed that the introduction of immune serum at the same time with virulent cholera spirilla into the peritoneum of guinea-pigs, prevented infection from taking place, and the spirilla were transformed into granular masses. He likewise showed this reaction to be specific, the serum of cholera-immune animals acting only on cholera vibrio, and hence he suggested using the serum as a means of diagnosis for the cholera vibrio and typhoid bacillus. Gruber about the same time made some studies upon the use of this serum property in differentiating bacteria, but it was considered as yet a property connected in some way with immunity.

In 1896 Widal and Grünbaum, working separately, developed what is now spoken of as the "Widal serum test," or "Widal reaction." It consists in testing a drop of blood of a patient suspected of having typhoid fever, by mixing a dilution of it with a drop of a fresh bouillon culture of typhoid bacilli, and examining the mixture in a hanging drop under the microscope. Within fifteen minutes to an hour the motility of the bacilli will cease, and they will have arranged themselves into clusters, as if stuck or glued together. If this reaction occurs within an hour, and with the proper dilution of the serum, the case is one of typhoid. Widal first used the serum of the blood; this has been modified so that even a drop of dried blood is sufficient. The method as applied in city laboratories is as follows: The physician is told to clean the finger of the patient with water (no germicides), and with a needle draw a drop of blood on to a piece of ordinary note-paper. This is then sent to the laboratory; the paper with the dried blood is soaked for a few minutes in a watch-glass containing 4 drops of clean water, thus obtaining a dilution of 1 : 5. One drop of this is then mixed with one drop of a bouillon culture of typhoid bacilli of about twenty-four-hours' growth, and examined under the microscope in the hanging drop. Weaker dilutions of the serum have been recommended (1 : 50), and this should be used in cases of doubt. So far, about 95 per cent. of the cases examined, and which clinically were considered typhoid fever, have given a positive reaction. It is not often present until the fifth day of the fever, and disappears usually within a year, though in some individuals it has been found ten years after an attack of the disease.

The agglutinating properties have been found in nearly all the secretions of the body—tears, urine, milk, pleuritic effusions, serous fluid from blisters, etc.

There is no relation between the reaction and the bactericidal power of the serum; the agglutination is not a destruction. The agglutinating power is active, though the blood be dried and sealed up for months. It seems to have no direct relation with the question of immunity, since it occurs at the height of the

disease, and intense agglutinating serum may be had in severe cases and in cases with relapses. A negative result does not exclude typhoid.

The test is *quantitative*—*i. e.*, it depends upon the dilution of the blood-serum, since the serum of healthy persons in strong dilution will cause agglutination and loss of motility.

The test must occur within a certain limit of time to be of value, since agglutination is liable to appear of itself with non-typhoid serums after a period of an hour.

A serum in a dilution of 1 : 30 causing complete clumping in half an hour is most likely typhoid.

The culture must be kept in a vigorous condition by frequent subplanting, and must be tested occasionally with normal serum. Cultures kept in an incubator for a long time tend to adhere in clumps naturally.

Sedimentation Test.—In a test-tube of bacterial emulsion and diluted serum, if the reaction is positive, there will be a flocculent precipitate or sediment at the bottom of the tube, the upper part remaining clear. Compared with normal serum, the cloudiness is diffuse.

As a clinical test of the disease it has considerable value, although operative at a time when other symptoms have developed sufficiently to determine the diagnosis.

Staining.—Colored with the ordinary anilin dyes, when they are *warmed*; since they are easily decolorized, acids should be avoided.

Gram's method is not applicable. Tissue sections stained as follows:

Alkaline methylene-blue.....	1 hour
Alcohol.....	5 seconds
Anilin-oil.....	5 minutes
Turpentine-oil.....	1 minute
Xylol and Canada balsam	

Such a specimen should first be examined with low power, to focus little colored masses, then examined with immersion lens; these masses will be found composed of bacilli.

Similar Bacteria.—The *Neapolitanus* bacillus of Emmerich, or *feces bacillus* of Brieger, resembles the typhoid bacillus in many ways, the colonies being the same and its structure similar. But the growth on potato is very different; a thick, yellow, pasty layer is formed thereon.

The *colon bacillus* not only resembles the typhoid germ in form, but also in some of the pathologic processes produced. For points of resemblance and difference see *Bacillus coli communis*.

Typhoid Bacilli in Water.—Although all evidence shows that the water-supply is a frequent source of infection, very few persons have ever isolated the typhoid bacillus from such an infected source. The earlier reports show that no account was taken of *Bacillus coli*, which is usually present in polluted waters. (See Water Analysis.)

Pathogenesis.—Lower animals do not have enteric fever, though their death has been caused by injection of the bacilli into the veins of the ear.

In man the bacillus has been found in the urine, blood, sputum, milk, intestinal discharges, roseolar spots, and in various organs, as spleen, liver, lymphatic glands, and intestinal villi.

It is found in secretions several days after the attack has subsided. It is found only in this disease, and regularly.

Typhoid Carriers.—Some individuals retain a culture of the bacilli in the gall-bladder for years, and manufacture, or at least expel, true virulent bacilli through the feces continually. Such persons have infected other individuals without suffering any inconvenience themselves. Some forms of chronic inflammation, as cholecystitis and appendicitis, have been caused by the typhoid bacillus.

Way of Infection.—The bacilli in the dejecta of the diseased person find their way into drinking-water, milk, or dirty clothes, and so into the alimentary tract of a person predisposed to the disease. Flies act as conveyors by infecting food. The bacilli enter the blood through the lymphatics, and so become lodged in various organs. They are quite resistant, living for some time in the soil and water, and are not affected, as other organ-

isms, by phenol. An epidemic has been traced to the eating of oysters taken from contaminated water.

Persistence in Water.—Franckland kept bacilli alive in water, sterilized by heat, seventy-five days; in filtered water at 19° C., five days; at 6° C., twelve days. In ordinary water they are likely to be destroyed in a few days by the overgrowth of other bacteria.

Products.—Brieger found a ptomain in the cultures, which he named *typhotoxin*, with the formula $C_9H_{17}NO_2$. It has no specific action. A toxalbumin insoluble in water has also been isolated, but, as experiment animals are immune to the disease, no definite actions have yet been determined.

The cultures, when old, show an acid reaction.

Vaccines.—Dead cultures of tested virulence are injected subcutaneously. This causes the blood to show agglutination reaction, increased opsonic index, and a heightened immunity to the disease. In several thousand inoculations observed by Wright it is claimed the disease was much lessened in severity and the tendency to infection much reduced.

Typhoid Bacilli in Blood.—Conradi, Busquet, Coleman, and Buxton, and others have found the bacilli in the blood of every patient by the following method. A mixture of ox-bile, 90 c.c., glycerin, 10 c.c., and peptone, 2 gm., is distributed into 20 c.c. flasks and sterilized. Ten cubic centimeters of blood is drawn from the elbow into a glass syringe and divided among three flasks. These are incubated, and in twenty-four hours litmus-lactose agar plates are inoculated on the surface by a stroke from the flasks. A growth is obtained in five or six hours.

If the growth is a bacillus which has not reddened the medium, it is tested for the Widal reaction with immune serum. The diagnosis has been made as early as the second day.

Paracolon or **paratyphoid bacilli** are members of the colon group recently described by Widal, Gwyn, Schottmüller, and others. They are of importance, since they produce fevers clinically resembling a mild form of typhoid, but which are

rarely fatal. They may be the sole cause of the disease, and also occur together with the typhoid bacillus in mixed and secondary infections. Morphologically, they resemble the typhoid bacillus, but differ from it culturally and give their own serum reactions with the blood of affected patients. They ferment glucose, but not lactose or saccharose; litmus milk at first becomes acid, but later grows alkaline and is not coagulated. On potato a slight visible growth occurs; indol is usually not formed. Typhoid serums do not agglutinate paracolon bacilli, and vice versâ; also different paracolon infections may not agglutinate each other. The *Bacillus enteritidis* of Gärtner is a related form.

Bacillus psittacosis is an allied form occurring in parrots, and producing hemorrhagic septicemia in them and other experiment animals. The disease is readily communicated to man from the affected birds, and causes, after ten days' incubation, a disease, the chief symptoms of which are fever, delirium, vomiting, diarrhea, and albuminuria, about a third of the cases ending fatally. The organism is agglutinated by strong dilutions of typhoid serum, but the clumping is incomplete and the bacillus differs further from the typhoid bacillus in its growth on potato and in the nature of the infection produced.

Bacillus Coli Communis (Escherich).—Found in human feces, intestinal canal of most animals, in pus and water.

Form.—Short rods with very slow movement, often associated in little masses resembling the typhoid germ, flagellated, not forming spores (Fig. 70).

Properties.—Does not liquefy gelatin, causes fermentation in saccharine solutions in the absence of oxygen, produces acid fermentation in milk, causes formation of indol in peptone solutions; its optimum temperature for growth is 37° C.

Growth.—On potato a thick, moist, yellow-colored growth; on agar a gray-white growth; on gelatin a growth similar to typhoid. It can also develop on phenol-gelatin, and withstands a temperature of 45° C. without its growth being destroyed.

Pathogenesis.—Inoculated into rabbits or guinea-pigs, death follows in from one to three days, the symptoms being those of

diarrhea and coma; after death tumefactions of Peyer's patches and other parts of the intestine; perforations into peritoneal cavity, the blood containing a large number of bacilli.

With the blood of immunized animals a serum reaction similar to that of typhoid fever may be obtained with cultures of colon bacilli. The colon bacillus is held responsible for most of the complications of typhoid fever, such as peritonitis, cholangitis, etc., by many writers.

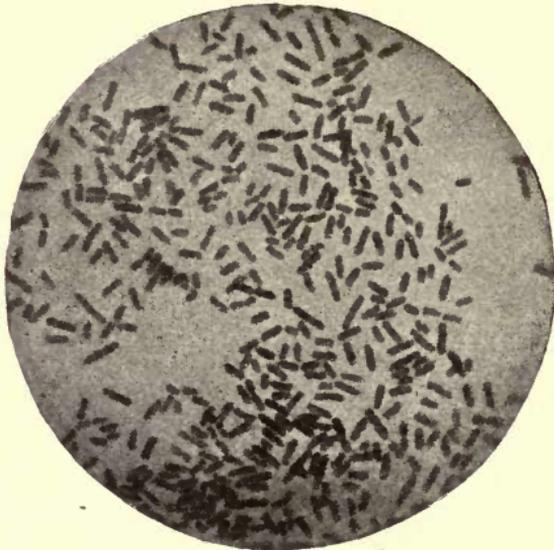


Fig. 70.—*Bacillus coli communis*, from an agar-agar culture ($\times 1000$) (Itzerott and Niemann).

Epidemics of a cholera or dysentery nature, called by Escherich *colitis contagiosa*, and due to infection of water and food, have been noted by a number of writers. The onset is very sudden and prostrating, though not fatal.

Many other forms of suppuration are associated with the presence of *Bacillus coli*.

Staining.—Ordinary stains; does not take Gram.

Site.—The bacillus has been found very constant in acute peritonitis and in cholera nostras. Its presence in water would indicate fecal contamination, as it is normally present in the intestine.

Points of Resemblance between Bacillus Typhi and Bacillus Coli Communis.—One, microscopic appearance; two, agar and gelatin cultures; three, sometimes growth on potato the same; four, staining peculiarities; five, resistance to phenol.

Points of Difference:

<i>Colon Bacillus.</i>	<i>Typhoid Bacillus.</i>
Less motile.	Actively motile.
Gelatin colonies develop more rapidly.	Develop more slowly.
Produces gas on dextrose or lactose media.	Does not.
Coagulates milk.	Does not.
Produces indol.	Does not.
Growth on potato visible.	Invisible.
Changes neutral red to yellow.	Does not reduce neutral red.

Differences are also noted in the growth on special media, such as those of Hiss and Elsner.

Varieties.—By some bacteriologists the following bacilli are all considered forms of the colon bacillus. *Bacillus lactis aërogenes* of Escherich; *B. cavicida*, of Brieger; *B. neapolitanus* of Emmerich; *B. enteritidis* of Gärtner, and, together with some other allied organisms, they are spoken of as the “colon group.”

Saccharolytic Bacteria.—These are organisms found in water that produce fermentation in sugar broth and form acids, but do not give the reaction for indol. Rivas has perfected some chemical tests that he claims distinguish this group from true *Bacillus coli*, but whether they possess any different pathogenic properties has not been determined.

***Bacillus Botulinus* (Van Ermengem).**—An anaërobic bacillus cultivated by Van Ermengem in 1896 from ham which had caused poisoning.

Form.—A large bacillus with rounded or spindle-shaped ends, and often with oval terminal spores, motile, with lateral flagella.

Staining.—Gram positive, easily stained with ordinary dyes.

Growth.—Strictly anaërobic. Forms abundant gas in glucose, gelatin, and liquefies cultures, producing butyric acid odor. Best temperature between 20° and 30° C.

Pathogenesis.—Produces a powerful toxin in the tissues, like the tetanus bacillus. This toxin may be present in the affected meat without causing decomposition, and thus give rise to poisoning.

Bacillus enteritidis of Gärtner has likewise been considered a cause of meat and sausage poisoning.

CHAPTER XIX

PATHOGENIC BACTERIA (Continued)

Spirillum Cholerae (Koch) (Comma Bacillus of Cholera).

—*Origin*.—Koch, as a member of the German expedition sent to India, in 1883, to study cholera, found this micro-organism in the intestinal contents of cholera patients, and by further experiments identified it with the disease.



Fig. 71.—Comma bacillus, pure culture ($\times 600$).

Form.—The spirillum as seen ordinarily appears as a short, arc-like body, about half the size of a tubercle bacillus, but when seen in large groups, spirals are formed, each little arc appearing then as but a segment, *a vibrio*; each arc is about three times as long as it

is broad, and possesses a flagellum at one or more, rarely both, ends.

Properties.—The spirilla are very motile; liquefy gelatin. They are easily affected by heat and dryness. Spores have not been found, though some (Hüppe) claim arthrospores, but these bodies represent only degenerative changes.

Growth.—At ordinary temperatures on all nutrient media

that have an alkaline or neutral reaction. They are facultative anaërobic.

Colonies, Gelatin.—After twenty-four hours, small white points which gradually come to the surface, the gelatin being slowly liquefied, a funnel-shaped cavity formed, holding the colony in its narrow part, at the bottom, and on the fifth day all the gelatin is liquid. If the colonies of three days' growth are placed under microscope, they appear as if composed of small bits of frosted glass with sharp irregular points.

Stab-culture.—After thirty hours a growth can be distinguished along the needle-track, and on the surface a little cavity is formed, filled by a bubble of air, and this liquefaction proceeds until, on the sixth day, it has reached the sides of the tube, tapering, funnel-shaped, to the bottom of the tube. After several weeks the spirilla are found in little collections at the bottom of the fluid gelatin. In eight weeks the bacilli have perished.

Agar.—Stroke cultures. A shiny white layer which lasts many months.

Potato.—A yellow, honey-like, transparent layer, if the potato is kept at animal heat.

Bouillon.—A wrinkled scum is soon formed in bouillon. The spirilla live well and grow in sterilized milk and sterilized water,

remaining virulent in the latter for many months. In ordinary water the bacteria present are destructive to the comma bacilli, and they die in a few days.

Dunham's Peptone Solution.—Useful for the development of nitrites and the indol reaction.

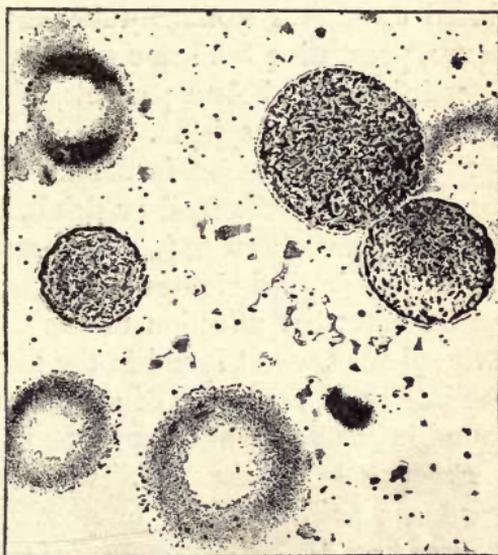


Fig. 72.—Cholera colonies after thirty hours ($\times 100$) (Fränkel and Pfeiffer).

Widal's serum test, as used in typhoid, is applicable in the diagnosis of cholera, using cholera cultures in place of the typhoid.

Staining.—They are colored well with watery anilin solutions. The flagella can be well seen by staining according to the flagella stain.

Pathogenesis.—Experiment animals are not subject to cholera Asiatica, but by overcoming two obstacles, Koch has produced choleraic symptoms in guinea-pigs. Nicati and Rietsch prevented peristalsis and avoided the acidity of the stomach-juices by direct injection into the duodenum, after tying the gall-duct. Koch alkalinizes the gastric juice with 5 c.c. of 5 per cent. solution of sodium carbonate, and then injecting 2 grams of opium tincture for every 300 grams of weight into the peritoneal cavity, paralyzes peristalsis. The cholera culture then introduced through a stomach-tube, the animals die in forty-eight hours, presenting the same symptoms in the appearance of the intestines as in cholera patients, the serous effusion containing great numbers of spirilla.

Manner of Infection in Man.—Usually through the alimentary tract, with the food or drink, the intestinal discharges of cholera patients having found entrance into the source of drinking water. Soiled clothes to fingers, fingers to the mouth, etc.; torpid catarrhal affection of the digestive tract predisposing. The spirilla are not found in the blood or any organ other than the intestines, the tissue of the small intestines. They are also found in the vomit and the intestinal contents.

Products.—"Cholera red." When chemically pure nitric or sulphuric acid is added to nutrient peptone cultures of the cholera bacillus, a rose-red color is produced. This will not take place with other bacilli unless *nitrous acid is present*. The cholera bacillus forms nitrites from the nitrates present in the media, and also indol. The mineral acid splits the nitrites, setting free nitrous acid, which, with the indol, forms the red reaction. This pigment has been isolated and extracted and called "*cholera red*." A ptomain, identical with cadaverin, and several other alkaloids, have been obtained from the cultures.

A toxalbumin and a toxic peptone have been isolated, but no special actions ascribed to them.

Detection of Cholera Organisms in Drinking-water.—When a few bacteria are supposed to be present in fecal matter or drinking-water, it is best to add a large quantity of the material (200 c.c. of drinking-water) to about 10 c.c. of bouillon or peptone-water, and place the mixture for twenty-four hours in an incubator, which will cause rapid reproduction, and then the organisms can be readily discovered.

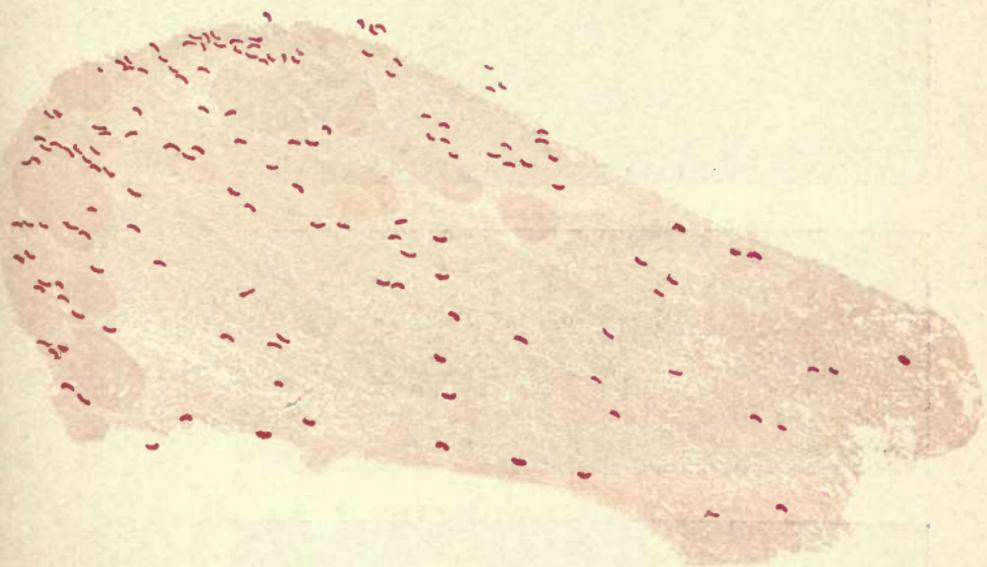


Fig. 73.—Comma bacillus in mucus, from a case of Asiatic cholera.

Protective Vaccines.—Virulent cultures killed by heat have shown protective power and were used extensively during an epidemic in Japan.

Haffkine has obtained a great reduction in mortality in cholera regions by the use of anticholera vaccines as protective and curative measures.

Cholera Immunity of Pfeiffer.—Intraperitoneal, subcutaneous, and intravenous injections of living or dead cholera bacteria cause a disease in animals similar to the cold stage of cholera. Death is the result of toxemia. If the animal lives,

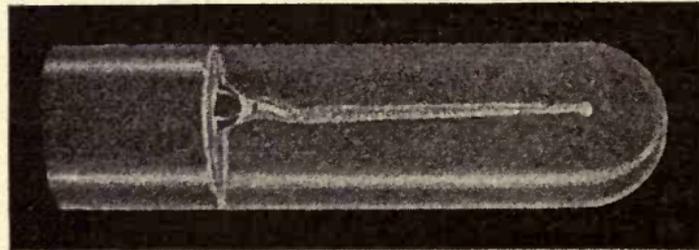


Fig. 74.—Cholera bacillus (forty - eight hours; 5 per cent. gelatin).

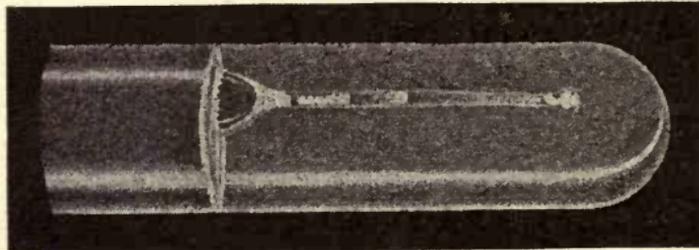


Fig. 75.—Cholera bacillus (sixty hours; 5 per cent. gelatin).

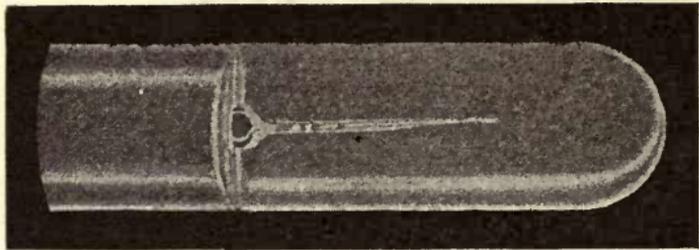


Fig. 76.—Cholera bacillus (seventy - two hours; 15 per cent. gelatin).

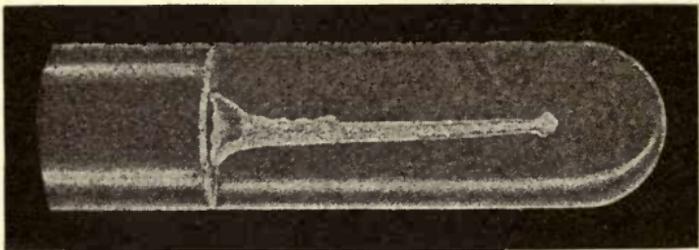


Fig. 77.—Deneke. Cheese bacillus (forty-eight hours; 5 per cent. gelatin).

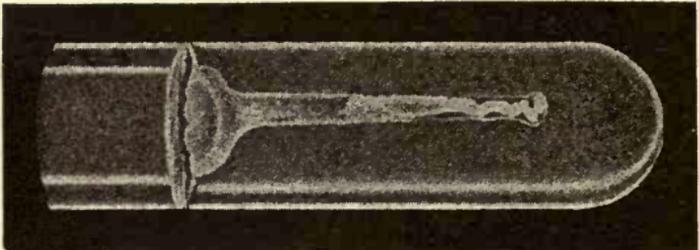


Fig. 78.—Deneke. Cheese bacillus (sixty hours; 5 per cent. gelatin).

Figs. 74-78.—Tube-cultures (from United States Government Report on Cholera.—Shakespeare).

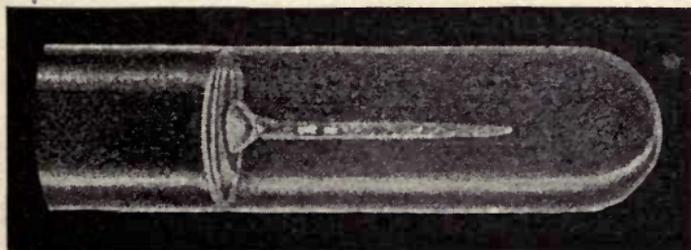


Fig. 79.—Deneke's Cheese bacillus (seventy-two hours; 15 per cent. gelatin).

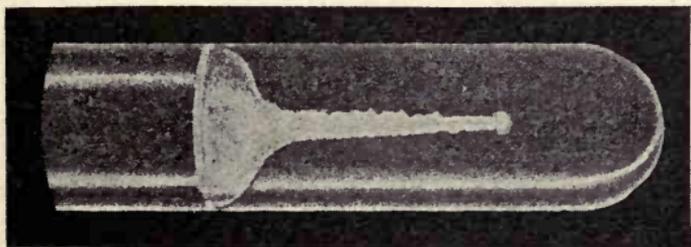


Fig. 80.—Finkler and Prior (forty-eight hours; 5 per cent. gelatin).

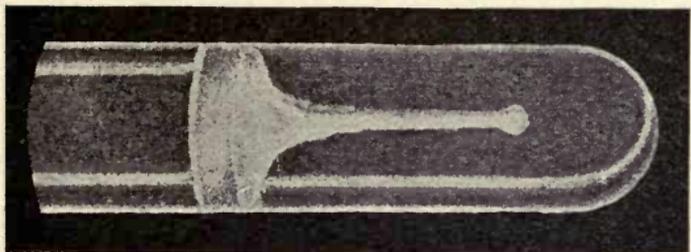


Fig. 81.—Finkler and Prior (sixty hours; 7 per cent gelatin).

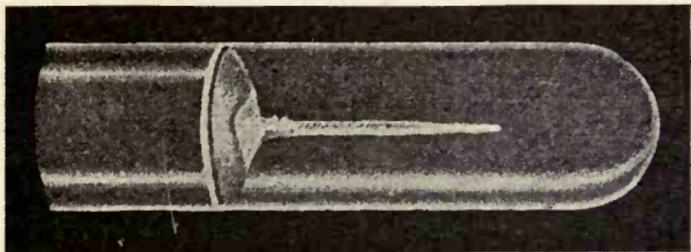


Fig. 82.—Finkler and Prior (seventy-two hours; 15 per cent. gelatin).

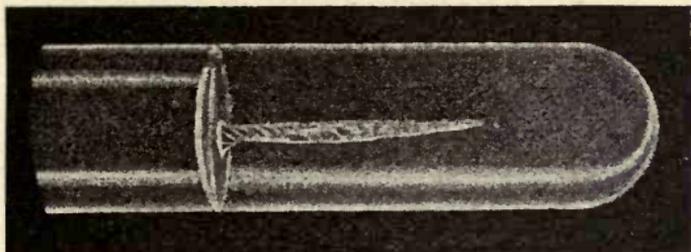


Fig. 83.—From glass-like colony—new bacillus (seventy-two hours; 15 per cent. gelatin).

Figs. 79-83.—Tube-cultures (from United States Government Report on Cholera.—Shakespeare).

the blood has protective properties of a specific nature; it has bactericidal properties against cholera vibrio, and by the injection of this serum into non-immune animals it renders them immune. The blood-serum of convalescents and cholera-vaccinated individuals contains the same bactericidal substances.

Serum therapy has not been successful.

Bacteria Similar to the Spirillum of Cholera.—**Finkler-Prior Vibrio, or Spirillum Finkleri.**—*Origin.*—Found in the intestinal contents of a patient suffering from cholera Asiatica in 1884, by Finkler and Prior, who thought it identical with the spirillum of cholera; it differs from it, however, in many ways, and has been found in healthy persons.



Fig. 84.—*Spirillum Finkleri* ($\times 700$) (Flügge).

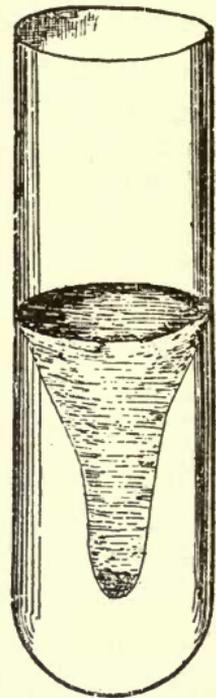


Fig. 85.—Stab culture (*Finkler-Prior*).

Form.—Somewhat thicker than the cholera vibrio, but forms the long spirilla less often. Has flagella.

Properties.—It is very motile. Liquefies gelatin in a short time.

Growth.—It grows quickly at ordinary room temperature. It is facultative aërobic.

Colonies on Gelatin Plates.—Round, finely granular colonies, which in twenty-four hours are ten times as large as the cholera colonies, and in forty-eight hours the whole plate is liquefied, it being then impossible to distinguish any separate colonies.

The microscopic appearances in no way resemble the cholera colony.

Stab-cultures.—The gelatin is liquefied from above downward, like a stocking in appearance, and in three days is completely liquid.

Potato.—At ordinary temperature a thick gray layer covering the whole surface.

Water.—It soon perishes in water.

Staining.—Ordinary anilin dyes.

Pathogenesis.—For man it has no specific action. If it is injected into guinea-pigs, prepared as described under the cholera bacillus, they die, the intestines having a foul odor, and the bacilli then found in great numbers.

Spirillum Tyrogenum (Deneke).—*Origin*.—In 1885 Deneke found in old cheese a spirillum very similar in appearance to the cholera spirillum.

Form.—The same as the cholera vibrio.

Properties.—Very motile, liquefy gelatin.

Growth.—They grow quicker than the cholera, and slower than the Finkler; they are also facultative aërobic.

Colonies.—At first resemble cholera colonies; have, however, a yellow-green iridescence and are more irregular; also grow more rapidly.

Stab-cultures.—A thick line along the needle-track and yellow colonies forming at the bottom; on the surface a bubble of air similar to the cholera. The gelatin is liquid in two weeks; on agar, rapid slimy yellowish growth.

Potato.—At brood-heat a thin yellow membrane, but not always constant. *Staining*, as cholera bacillus.

Pathogenesis.—When injected into animals prepared as for the cholera bacillus, a certain number die.

Vibrio Metchnikovi (Gamaleia).—*Origin*.—In the intestines of fowls suffering from a gastro-enteritis, common in Russia. Gamaleia found a spirillum which bears so close a resemblance to the cholera bacillus, both in form and growth, that it cannot be distinguished by these characteristics alone.

Form.—As cholera bacillus.

Growth.—Two kinds are found on the gelatin plate—one that is identical in appearance with the cholera colony, the other more liquefying, resembling the Finkler spirillum. If now a second plate be inoculated from either one of these forms, both kinds again are found grown, so that it is not a mixture of two bacilli; on agar, brownish-yellow growth.

Stab-culture.—Similar to the cholera growth, a trifle faster in growing. *Staining*, as cholera.

Pathogenesis.—To differentiate it from cholera, these bacilli, when injected into animals, prove very fatal, and no especial precautions need be taken to make the animal susceptible. In the pigeon, guinea-pig, and chicken a hemorrhagic edema and a septicemia is produced which has been called "*Vibrion septicemia*." The blood and organs contain the spirilla in great numbers.

Products.—The nitrites are formed in cultures just as with cholera bacillus, and the red reaction produced when mineral acids are added to gelatin cultures. Certain products are formed which, when injected, give immunity. The cultures are first heated for one-half hour at 100° C., which destroys the germs, and then this sterilized product injected (5 c.c. of a five-days'-old sterilized culture). In two weeks 1 to 2 c.c. of the infected blood can be injected without causing any fatal result.

Many other spirilla resembling the spirillum of cholera have been isolated from drinking-waters in the past few years, and some bacteriologists are inclined to consider them as varieties of the true cholera spirillum, which require only certain conditions to make them pathogenic. Among these, besides those already described, are *Spirillum berolinense*, *S. dunbarii*, *S. danubicum*, *S. of wernicke*, *S. bonhoffii*, *S. weibeli*, *S. schuykilliensis*, *S. milleri*, *S. aquatilis*. The last two are non-pathogenic for experiment animals.

Bacteria of Pneumonia.—Two forms of bacteria have been found in this disease, and thought at different times to be the cause of the same.

Neither one of them is constant in pneumonia; and since

many other pathologic processes have shown them, they can hardly be set down as the sole cause of pneumonia.

Klebs in 1875 called attention to the presence of bacteria in pneumonia, and in 1882 Friedländer developed a bacillus from the lung tissue of a pneumonic person, which he thought was a coccus, and called it pneumococcus.

In 1886 A. Fränkel and Weichselbaum proved that this organism was not constant—in fact, was rare.

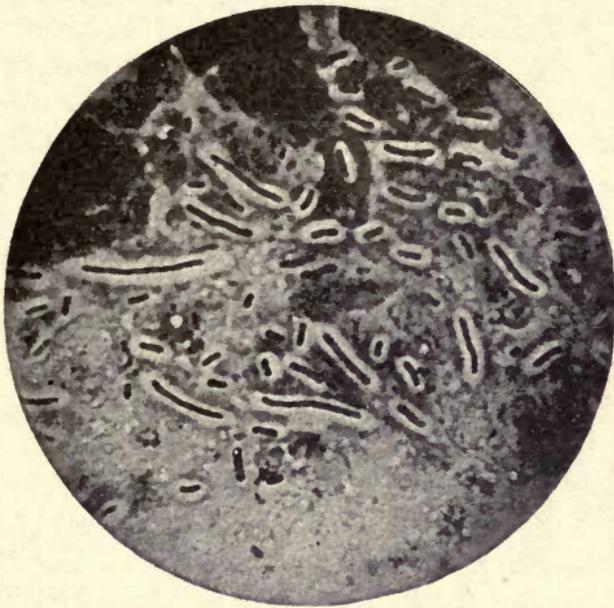


Fig. 86.—*Bacillus pneumoniae* of Friedländer, from the expectoration of a pneumonia patient ($\times 1000$) (Fränkel and Pfeiffer).

A. Fränkel obtained in the majority of cases of pneumonia an organism that he had described in 1884 under the name of sputum-septicemia micrococcus.

Weichselbaum called it *Diplococcus pneumoniae*, and believed it to be the real cause of pneumonia. It has been found in many other serous inflammations, and also in the mouths of healthy persons. It is the generally accepted organism of the disease, and can be isolated from nearly all cases of acute croupous pneumonia. It is found in about three-quarters of all cases of pneumonia.

Streptococcus pyogenes and *Staphylococcus pyogenes aureus* have been found in some cases.

Pneumobacillus (Pneumococcus) (Friedländer).—*Origin.*—In the lung of a croupous pneumonia person, by Friedländer, in 1882.

Form.—Small, almost oval-shaped rods, nearly as wide as they are long; often in pairs, they were at first believed to be cocci. In bouillon cultures the rod form becomes more visible.

In tissues each bacillus is surrounded by a faint capsule; but not around those developed in artificial cultures. Spores have not been found.

Properties.—They are immobile; do not liquefy gelatin. A gas is produced in gelatin cultures. Ferment sugar solutions.

Growth.—Grows rapidly on all media at ordinary temperature; is facultative aërobic.

Colonies.—On gelatin plates. Small white round colonies, reaching the surface in the course of three or four days; appearing then as little buttons, with a porcelain-like shimmer, the edges smooth.

Stab-culture.—A growth along the needle-track, but on the surface a button-like projection, which gives to the growth the appearance of a nail driven into the gelatin, its head resting on the surface; therefore such cultures are called "*nail cultures.*" (See Fig. 87.) Old cultures are colored

brown and contain bubbles of gas; on agar and blood-serum, a thick white viscid growth.

Potato.—A yellow, moist layer in a few days at brood-heat. Gas-bubbles develop.

Staining.—The ordinary anilin stains. The sections do not take Gram's method: are, therefore, not suited for double staining.

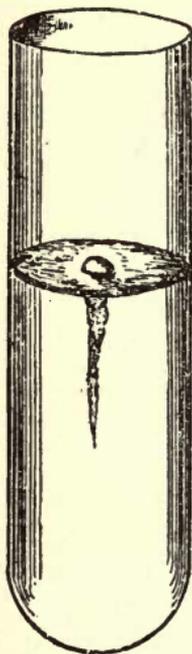


Fig. 87.—Bacillus of pneumonia. Stab-culture (nail culture).

Capsule.—Stained as follows:

Cover-glasses:

1. Acetic acid, two minutes.
2. Allow acetic acid to dry by blowing air upon it through a glass tube.
3. Saturated anilin-water. Gentian-violet, ten seconds.
4. Rinse in water. Mount in Canada balsam.

For sections:

1. Stain for twenty-four hours in the following, warmed:

Concentrated alcoholic gentian-violet.....	50.0
Aqua.....	100.0
Acetic acid.....	10.0.—M.

2. Rinse in 1 per cent. acetic acid.
3. Alcohol to dehydrate. Mount in balsam.

The capsule will be found stained a light blue, the bacillus a deep blue. (See also the Capsule Stain of Hiss, p. 35, and that of Burger.)

Pathogenesis.—Animals are not affected unless the culture is injected intrapleural.

Pneumobacillus of Fränkel (A. Fränkel and Weichselbaum).—*Synonyms*.—Pneumococcus; diplococcus of pneumonia; micrococcus of sputum septicemia; Micrococcus pasteurii; Diplococcus lanceolatus.

Origin.—A. Fränkel found it in the sputum of pneumonic patients, thinking it at first to be the micrococcus of sputum septicemia; later he believed it to be the cause of pneumonia.

Form.—They were at first called oval cocci, but they are now known to be rod-shaped, being somewhat longer than broad, varying, however, much in size and shape. Usually found in pairs, sometimes in filaments of three and four elements. In the material from the body a capsule surrounds each rod. In the artificial cultures this is not found.

Properties.—They are without self-movement; do not liquefy gelatin. There are no spores.

Growth.—Grow only at high temperature— 35° C.; are facultative anaërobic. The culture-media must be slightly alkaline; the growth is slow.

Colonies on Gelatin Plates.—Since the temperature must be somewhat elevated, the gelatin media need to be thicker than usual (15 per cent. gelatin), in order to keep it solid, and a temperature of 24° C. used. Little round white colonies, somewhat granular in the center, growing very slowly.

Stab-cultures.—Along the needle-track small separate white granules, one above the other, like a string of beads.

Stroke-culture.—On agar, transparent, almost invisible little drops, resembling dew moisture.

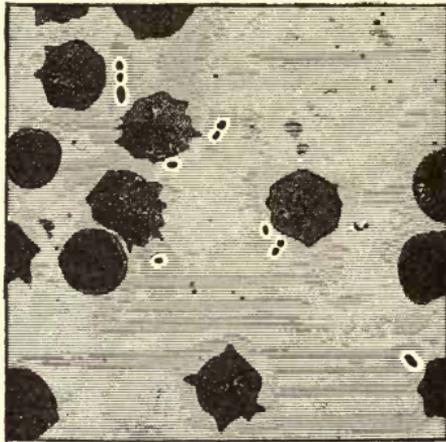


Fig. 88.—Bacillus of pneumonia in blood of rabbit ($\times 1000$) (Fränkel and Pfeiffer).

Bouillon.—They grow better here than in the other media, remaining alive a longer period of time.

Blood-serum and Agar.—A good growth on blood-serum or blood-agar.

Staining.—Takes Gram's method and the other anilin stains very readily. The capsule stained the same way as that of the *Friedländer bacillus*.

Pathogenesis.—Rabbits and guinea-pigs, if subcutaneously injected, die in the course of a couple of days with septicemia (0.1 c.c. of a fresh bouillon culture suffices).

Autopsy shows greatly enlarged spleen and myriads of bacilli in the blood and viscera, the lungs not especially affected. If injected into the trachea, a pneumonia occurs. In man they are found in 90 per cent. of croupous pneumonia, and usually only during the existence of the rusty sputum, *i. e.*, the first stage.

The bacilli have also been found in pleuritis, peritonitis, pericarditis, meningitis, and endocarditis. They stand in some intimate relation to all infectious inflammations of the body. Their presence in healthy mouth secretion does not speak against this, it requiring some slight injury to allow this ever-present germ to develop into disease.

Antitoxin of Pneumonia (Klemperer).—The injection of very diluted cultures of the virulent bacilli intravenously has produced an immunity in rabbits and guinea-pigs. The serum of such artificially immune animals when filtered through a Chamberland filter and injected into a rabbit suffering with pneumonia, cured the same; or when injected into a susceptible animal, produced in it immunity very quickly. This principle is ascribed to an antitoxin formed in the tissues by the diluted proteids, and this antitoxin neutralizes the toxicity of the strong virus. The attempt to treat with antitoxins has been abandoned, the disease ending so rapidly by crisis that it is difficult to trace any curative effect from remedies.

Opsonins.—The opsonic index in the studies so far made does not show any marked change from the normal.

Virulent pneumococci are insusceptible to phagocytosis.

Bacillus of Rhinoscleroma (Frisch, 1882).—It was found in the tissue of a rhinoscleroma, but resembles the Friedländer bacillus in nearly every respect, and as the disease rhinoscleroma is not reproduced by the inoculation of the bacillus in animals, it can be considered identical. The growth, cultures, and properties are the same as the pneumobacillus of Friedländer.

Diplococcus Intracellularis Meningitidis (Weichselbaum).—*Origin.*—Found by Weichselbaum in epidemic cerebrospinal meningitis in 1887.

Form.—A small coccus occurring in pairs, flattened against each other, and contained within the leukocytes.

Properties.—Ferments sugars, with acid production.

Growth.—Best in blood-agar, serum-agar, and ascitic glucose-agar at body temperature in twenty-four hours.

Colonies.—Circular discs, almost transparent under low power; margins smooth.

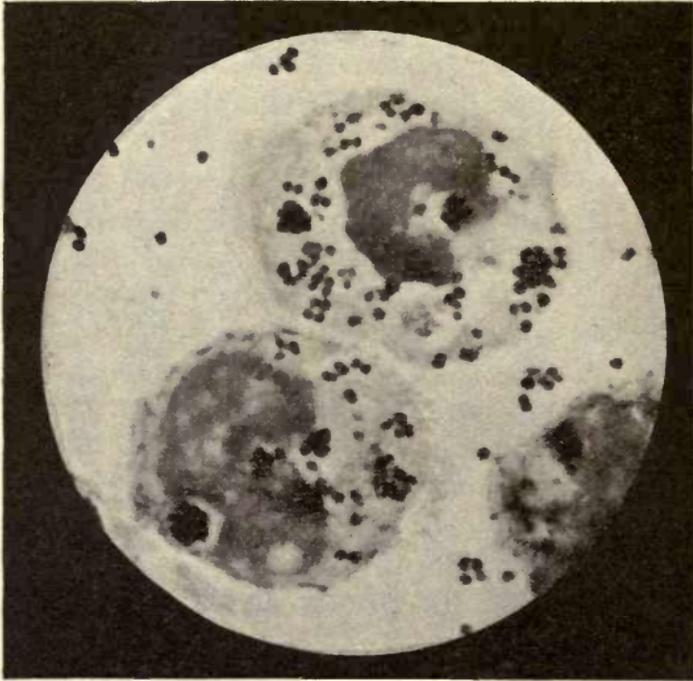


Fig. 89.—*Diplococcus intracellularis meningitidis* in leukocytes. Cover-glass preparation from peritoneal exudate in a guinea-pig ($\times 2000$) (Wright and Brown).

Stain.—With basic anilin. Gram negative, resembling gonococcus.

Pathogenesis.—Causes epidemic cerebrospinal fever, probably by infection through the nasopharynx; the organism is found in the spinal fluid and in other inflammatory exudates, and can be examined in fluid by lumbar puncture.

Ordinary laboratory animals immune, but Flexner has succeeded in inoculating monkeys.

Agglutination.—On the fourth day; in dilution of 1 : 50 agglutination is bad.

Protective Serum.—Flexner has been able to obtain an anti-toxin from monkey serum that has therapeutic properties in man.

Micrococcus Tetragenus (Koch; Gaffky).—*Origin.*—Koch found this microbe in the cavity of a tuberculous lung. Gaffky, in 1883, studied its pathogenic actions and gave it the name it now bears.

Form.—Cocci which are gathered in the tissues in groups of four, forming a square—a tetrad. (See Fig. 90.) In artificial

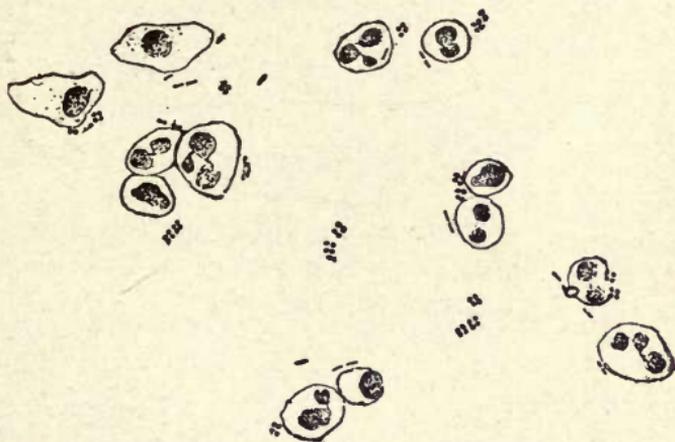


Fig. 90.—Micrococcus tetradenus in sputum (tubercle bacillus also).

culture sometimes found in pairs. A capsule of light, gelatinous consistence surrounds each tetrad.

Properties.—They are immobile; do not liquefy gelatin.

Growth.—They grow well on all nutrient media at ordinary and brood temperatures; are facultative aërobic. They grow slowly.

Colonies in gelatin plates. In two days, little white spots, which, when on the surface, form little elevations of a porcelain-like appearance; under low power they are seen very finely granulated.

Stab-culture.—Small round separated colonies along the

needle-track, and on the surface a button-like elevation—a form of “nail culture.” (See Fig. 91.)

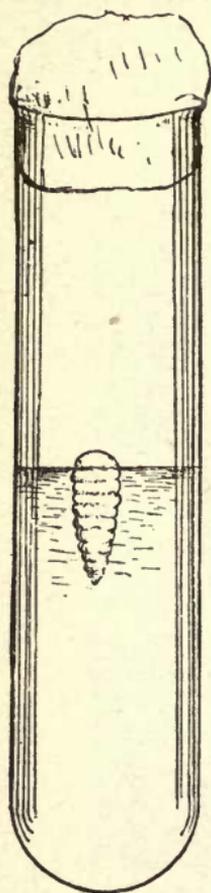


Fig. 91.—Stab-culture. *Micrococcus tetragenus*.

Potato.—A thick, slimy layer which can be loosened in long shreds.

Staining.—Colored with the ordinary anilin stains. Gram's method also applicable.

Pathogenesis.—White mice and guinea-pigs die in a few days of septicemia when injected with the tetragenus cultures, and the micrococcus is then found in large numbers in the blood and viscera. Field-mice are immune.

In the cavities of tuberculous lungs, in the sputum of phthisical and healthy patients, it is often found, but what action it has upon man has not yet been determined.

Capsule Bacillus (Pfeiffer).—*Origin*.—Stringy exudate and blood of a dead guinea-pig.

Form.—Thick little rods, sometimes in long threads. Large oval capsules in the stained preparations (Fig. 92).

Properties.—Immotile, not liquefying, an odorless gas in gelatin cultures.

Growth.—At ordinary temperatures, rapidly; facultative anaërobin.

Gelatin Plates.—Oval points, and like a porcelain button on the surface.

Stab-cultures.—Like the pneumonia bacillus of Friedländer.

Potato.—Abundant growth, yellow color and moist, coming off in strings.

Staining.—Hot fuchsin colors the capsule intensely; carefully decolorizing with acetic acid, the capsules are red or light violet around the deeply tinged bacillus. Gram's method not applicable.

Pathogenesis.—Subcutaneously injected in mice, they die in forty-eight hours. Rabbits die when a large quantity is injected

into the circulation. The blood and juices have a peculiar stringy, fibrinous consistence.

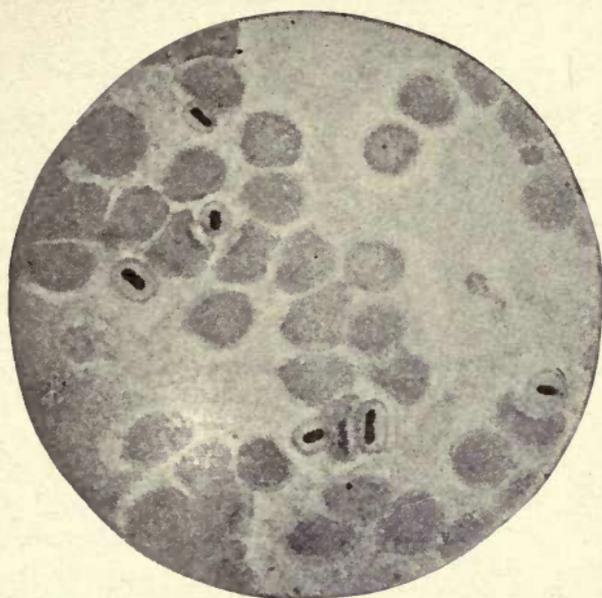


Fig. 92.—Pfeiffer's capsule bacillus in blood ($\times 1000$) (Fränkel and Pfeiffer).

Bacillus of Influenza (Pfeiffer, 1892).—*Origin.*—A small bacillus, about one-half the size of the bacillus of mouse septicaemia, and arranged in chain form. It develops upon blood-serum agar. It is aërobic, without movement; does not take the Gram stain (Fig. 93).

Stain.—It is best stained with diluted carbol-fuchsin, the contrast-stain being Löffler's methylene-blue.

Growth.—Upon glycerin-agar, over which a drop of blood has been spread, in an incubator at the end of twenty-four hours, a very delicate growth occurs, which resembles condensed moisture.

Pathogenesis.—It is found in the sputum and in the bronchial nasal secretions and blood of influenza patients. It has been transmitted to monkeys; other animals are not susceptible. It has never been found outside the body. Its resistance is very feeble; in water, the bacilli die in twenty-four

hours, but sputa containing the germs may be ejected for days and weeks. Influenza bacilli are found accompanying bron-

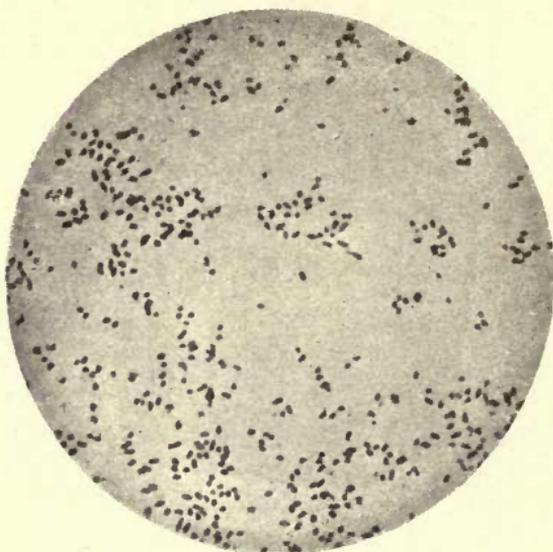


Fig. 93.—*Bacillus influenzae*, from a gelatin culture ($\times 1000$) (Itzerott and Niemann).

chopneumonia, tuberculosis, meningitis, and other inflammations.

Microörganisms of Suppuration.—The suppuration of wounds is due to the presence of germs. The knowledge of this fact is the basis of the antiseptic treatment in surgery; for when the microbes can be destroyed or their entrance prevented, the wounds are made clean and kept without suppurating. Various forms of bacteria have been found in septic processes, and the formation of *pus* cannot be ascribed to any particular one alone; some, more common than others, are found in nearly all forms of suppuration; others give rise to special types.

Wounds are often irritated by foreign bodies and chemicals, and a discharge occurs in them even when every aseptic and antiseptic precaution has been taken; but such a discharge is free from bacteria, and no more like pus than a benign growth is like a malignant one.

Streptococcus Pyogenes (Rosenbach); Streptococcus erysipelatis (Fehleisen).—*Origin.*—Fehleisen discovered this microbe in the lymphatics of the skin in erysipelas, and he thought it the cause of the same. Under the name *Streptococcus pyogenes*, Rosenbach described an identical coccus which has been found in nearly all suppurative conditions.

Form.—Small cocci singly and in chain-like groups. Spores have not been found; though it is supposed, because of their permanency, that spores are present.

Properties.—They are immotile; do not liquefy gelatin.



Fig. 94.—*Streptococcus pyogenes*: culture upon agar-agar two days old (Fränkel and Pfeiffer).

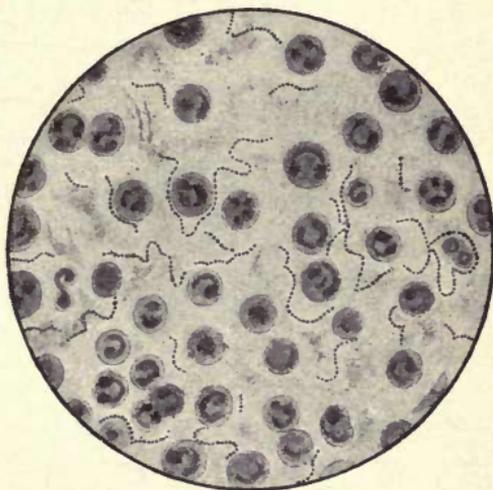


Fig. 95.—*Streptococcus pyogenes* (Jakob).

Growth.—They grow slowly, usually on the surface, and best at higher temperatures.

Colonies.—In three days a very small grayish speck, which hardly ever becomes much larger than a pin-head; under microscope, looking yellowish, finely granular, the edges well defined.

Stab-cultures.—Along the needle-track little separated col-

onies, like strings of beads, which after a time become one solid white string.

Stroke-culture on Agar.—Little drops, never coalescing, having a bluish tint, very transparent.

Potato.—No apparent growth.

Bouillon.—At 37° C. clouds are formed in the bouillon, which then sink to the bottom, and long chains of cocci found in this growth.

Staining.—Easily colored with the ordinary stains. Gram's method is also applicable.

Pathogenesis.—Inoculated subcutaneously in the ear of a rabbit, an erysipelatous condition develops in a few days, rapidly spreading from point of infection.

In man, inoculations have been made to produce an effect upon carcinomatous growths and *erysipelas* was always produced. When it occurs upon the valves of the heart, *endocarditis* results. *Puerperal fever* is caused by the microbe infecting the endometrium, the *Streptococcus puerperalis* of Fränkel being the same germ.

In scarlatina, variola, yellow fever, cerebrospinal meningitis, and many similar diseases, the microbe has been an almost constant attendant. It is often associated with the diphtheria bacillus in true diphtheria, and is the cause of many of the diphtheritic affections of the throat in which the diphtheria bacillus is absent.

An antistreptococcic serum has been used as a curative agent in puerperal fever, scarlatina, and other diseases supposed to be due to this germ.

A mixture of a culture of pyogenes and prodigiosus has been used as an injection, with apparent benefit, in inoperable cases of sarcoma, and is known as Coley's fluid.

Staphylococcus Pyogenes Aureus (Rosenbach).—*Origin*.—Found commonly in pus (80 per cent. of all suppurations), in air, water, and earth; also in sputum of healthy persons.

Form.—Micrococci in clusters like bunched grapes, hence the name *staphylo*, which means grape. They never form chains.

Spores have not been found, though the cocci are very resistant.

Properties.—Without movement; liquefying gelatin. It gives rise to an orange-yellow pigment in the various cultures.

Growth.—It grows moderately fast at ordinary temperature, and can live without air, a facultative aërobin and anaërobin.

Colonies on Gelatin.—On second day small dots on the surface, containing in their center an orange-yellow spot. The gelatin all around the colony is liquefied; the size is never much greater than that attained the second day.

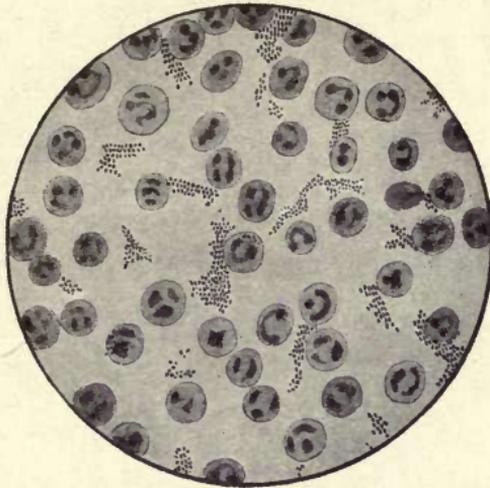


Fig. 96.—*Staphylococcus pyogenes albus* (Jakob).

Colonies on Agar.—The pigment remains for a long time.

Stab-culture.—At first, gray growth along the track, which, after three days, has settled at the bottom of the tube in a yellow, granular mass, the gelatin being all liquid.

Stroke-culture on Agar.—The pigment diffused over the surface where the growth is, in moist masses.

Potato.—A thin white layer which gradually becomes yellow and give out a doughy smell.

Staining.—Very readily colored with ordinary stains; also with Gram's method.

Pathogenesis.—When rabbits are injected with cultures of this microbe into the knee-joint or pleura, they die in a day. If injected subcutaneously, only a local action occurs, namely, abscesses.



Fig. 97.—Stab-culture. *Micrococcus pyogenes aureus.*

If directly into circulation, a general phlegmonous condition arises, the capillaries become plugged with masses of cocci, infarcts occur in kidney and liver, and metastatic abscesses form in viscera and joints. Garré, by rubbing the culture on his forearm, caused carbuncles to appear.

Several varieties of the pyogenic staphylococci are recognized according to their color-producing properties and slight variations of growth. Of these, the *Staphylococcus pyogenes aureus* is the most virulent, and is considered the type of the group. They are always present on the surface of the body, beneath the nails, in the nose and mouth, in the dust of streets, and on the floor of houses.

Staphylococcus pyogenes albus differs from the preceding only in the absence of pigment and in its slight virulence. Welch describes a variety constantly found both on the skin and in its deeper layers, which he calls the *staphylococcus epidermidis albus*.

***Micrococcus Pyogenes Citreus* (Passet).**—This liquefies gelatin less rapidly than the *pyogenes aureus*, and forms a citron-yellow pigment instead of the orange-yellow of the *aureus*.

***Micrococcus Cereus Albus* (Passet).**—Differs from the *pyogenes albus* in the form of colony. A white, shiny growth, like drops of wax; hence the name, *cereus*.

***Micrococcus Cereus Flavus* (Passet).**—A lemon-yellow colored growth after a short time, otherwise not differing from *cereus albus*.

***Micrococcus Pyogenes Tenuis* (Rosenbach).**—*Origin.*—Found in the pus of large inclosed abscesses.

Form.—Cocci, without any especial arrangement.

Properties.—Not much studied.

Growth.—Cultivated on agar, it forms clear, thin colonies; along the needle-track an opaque streak, looking as if varnished over.

Bacillus Pyocyaneus (Gessard).—*Synonyms.*—*Bacterium aëruginosum*; *Bacillus fluorescens* (Schröter); the bacillus of bluish-green pus.

Origin.—Found in 1882 in green pus in pyocymia.

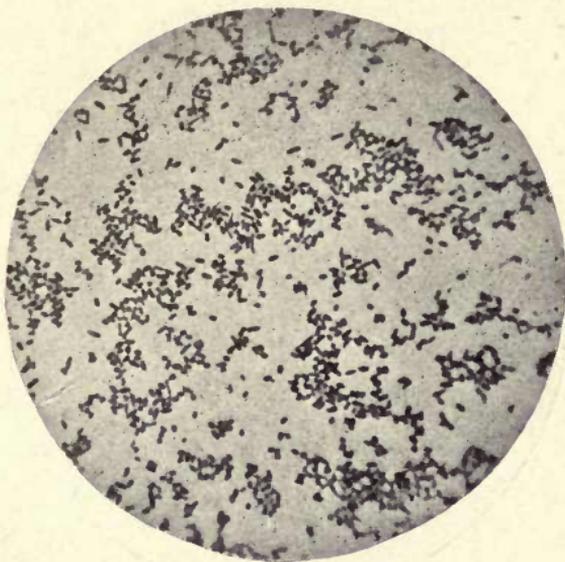


Fig. 98.—*Bacillus pyocyaneus*, from an agar-agar culture ($\times 1000$) (Itzerott and Niemann).

Form.—Small slender rods with rounded ends, easily mistaken for cocci. Often in groups of four and six, without spores.

Properties.—Very motile; liquefy gelatin rapidly; a peculiar sweetish odor and a blue pigment are produced in the cultures.

Growth.—Develops readily at ordinary temperature, growing quickly and mostly on the surface; it is aërobie. *Colonies on gelatin plate:* In two or three days a greenish iridescence appears over the whole plate, the colonies having a funnel-shaped lique-

faction, and appearing, under low power, when still young, as yellowish green, the periphery being granulated.

On agar: A bright green at first, causing fluorescence; then later a blue pigment.

Stab-cultures.—Mainly in upper strata, the liquefaction funnel shaped, the growth gradually settling at the bottom, a rich green shimmer forming on the surface, and the gelatin having a deep fluorescence.

Potato.—The potato is soaked with the pigment, a deep fold of green occurring on the surface.

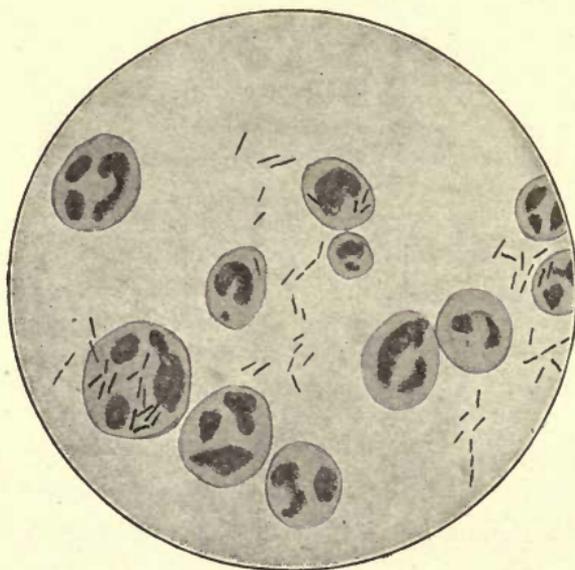


Fig. 99.—Koch-Weeks' bacillus from conjunctival exudate at third day of epidemic conjunctivitis (Boston).

Staining.—With ordinary anilin dyes.

Pathogenesis.—When animals are injected with fresh cultures in the peritoneal cavities or cellular tissues, a rapidly spreading edema with general suppuration develops. The bacilli are found in the viscera and blood.

If a small quantity is injected, a local suppuration occurs, and if the animal does not die, it then can withstand large quantities. It is immune.

The Pigment.—*Pyocyanin.*—When the pus, bandages, and

dressings containing the *Bacillus pyocyaneus* are washed in chloroform, the pigment is dissolved and crystallizes from the chloroform in long needles. It is soluble in acidulated water, which is turned red thereby, and when neutralized, the blue color returns. It has no pathogenic action. It is an aromatic compound. The bacillus has no especial action on the wound, and is found sometimes in perspiration of healthy persons.

Bacillus Pyocyaneus β (Ernst).—A bacillus found in grayish, pus-colored bandages.

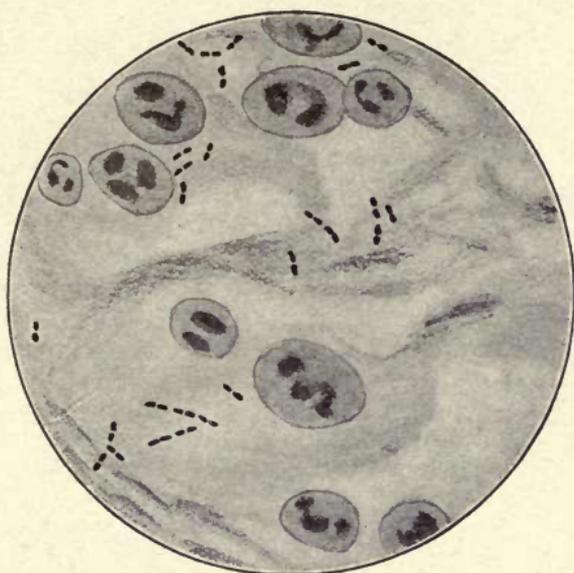


Fig. 100.—Morax-Axenfeld diplobacillus from conjunctival exudate during course of subacute conjunctivitis (obj. B. and L., one-twelfth oil-immersion) (Boston).

The only especial difference between this and the above is the formation of brownish-yellow pigment instead of *pyocyanin*. The form and appearance of cultures otherwise the same.

Koch-Weeks' Bacillus (1883-87).—Cause of epidemic conjunctivitis, or "pink eye"; found in the secretion.

Form.—Very minute bacillus, resembling the influenza bacillus; non-motile. (See Fig. 99.)

Growth.—They grow best on blood-serum agar, but very sparsely in minute transparent colonies; non-liquefying.

Stains.—With carbol-fuchsin, and is often intracellular. Does not take Gram.

Pathogenesis.—Very contagious, found in 10 per cent. to 20 per cent. of all cases of conjunctivitis. Not infectious for lower animals, and not causing any other form of disease.

Morax-Axenfeld Diplobacillus of Conjunctivitis.—This bacillus is found in the greater number of cases of conjunctivitis.

Form.—A short, plump bacillus, usually in pairs and chains of pairs. Non-motile (Fig. 100).

Growth.—With difficulty in blood-serum agar, it forms small pitted colonies or lacunæ; liquefies.

Staining.—Does not take Gram, but stains readily.

Non-pathogenic for lower animals.

Micrococcus Gonorrhœæ (Gonococcus) (Neisser).—In 1879 Neisser demonstrated the presence of this germ in the secretion of specific urethritis.

Form.—Cocci, somewhat triangular in form, found nearly always in pairs, the base of one coccus facing the base of the other and giving the appearance of a Vienna roll, hence the German name, *semmel* (roll), form. Four to twelve such pairs are often found together. Immotile (Fig. 102).

Culture.—On gelatin-agar or potato they do not grow, and only upon human blood-serum have they given any semblance of a growth. The temperature must be between

33° and 37° C., and the growth occurs very slowly and sparsely.

Method of Cultivation (Wertheim).—Gonorrhœal pus is mixed in a test-tube with liquid human blood-serum of 40° C. temperature, and two dilutions are made with blood of the same temperature. An equal quantity of 2 per cent. agar solution is now poured into each tube, and three glass dishes are covered at once with this mixture. After being in the brood-oven for twenty-four hours, colonies can be discovered.

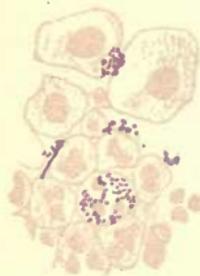


Fig. 101.—
Gonococci in gonorrhœal pus. Anilin methyl-violet (× 650).

In three days a very thin, almost invisible, moist yellowish growth, seemingly composed of little drops.

Under low power small processes are seen shooting out from the smooth border.

It requires then to be transferred to fresh media, as the culture quickly perishes.

Cultivation has also occurred on acid gelatin, gelatin containing acid urine, in acid urine itself, and albuminous urine with agar.

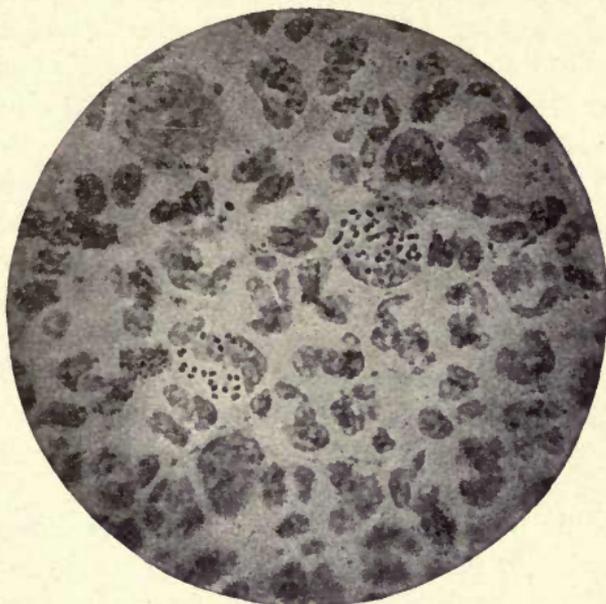


Fig. 102.—Gonococcus in urethral pus ($\times 1000$) (Fränkel and Pfeiffer).

In septicemic cases the gonococcus has been isolated from the blood direct by drawing 5 to 10 c.c. from a vein and adding it in equal parts to melted agar. The mixture is poured into Petri dishes and developed in the incubator at 37° C.

Staining.—Colored easily with all ordinary anilin stains.

Gram's method is not applicable, this being one of its main diagnostic features.

The following method is recommended by Neisser.

The cover-glasses, with some of the urethral discharge smeared upon them, are covered with a few drops of alcoholic

solution of eosin, and heated for a few minutes over the flame. The excess of the dye is removed with filter-paper, then the cover-glass placed in concentrated methylene-blue (alcoholic solution) for fifteen seconds, and rinsed in water.

The gonococci are dark blue, the protoplasm of the cell pink, and the nucleus a light blue, the gonococci lying in the protoplasm next to the nucleus (Fig. 101).

Other bacteria are similar to the gonococci in form; they are distinguished from the gonococcus in that they are positive with Gram's method, whereas the micrococcus of gonorrhoea is not. The points on which the diagnosis is to be made are the characteristic biscuit shape, the intracellular position of the organism, and its failure to stain with Gram.

Pathogenesis.—The attempts to infect the experiment animals with gonorrhoea have so far been without success. In man, upon a healthy urethra, a specific urethritis was produced with even the twentieth generation of the culture. *Gonorrhoeal ophthalmia* contains the cocci in great numbers, and endocarditis and gonorrhoeal rheumatism are said to be caused by the cocci.

The micrococci have been found long after the acute attack, when only a very slight oozing remained, and the same were very virulent.

The specific inflammations of the generative organs of the female are due to this organism gaining entrance through the vagina. It is found chiefly in the superficial layers of the mucous membrane.

A temperature of 40° C. for twelve hours destroys the gonococci.

Gonotoxin.—A toxin has been isolated which causes fever, loss of weight, and finally death. The urethra is not immunized by repeated injections. In man the toxin causes painful indurations, lasting several days.

Similar Bacteria Found in the Urethra and Vagina.—**Micrococcus Citreus Conglomerata (Bumm).**—Similar to the gonococci in form, they are, however, *easily cultivated*, and form yellow colonies which dissolve the gelatin and grow rapidly; the surface of the gelatin is at first moist and shiny, but

later on wrinkled. Colored with Gram's method, and have no special pathologic action. Found in the air and gonorrhoeal pus.

Diplococcus Albicans Amplus (Bumm).—In vaginal secretion. The diplococci are much larger than the gonococci, but similar in form. They are also cultivated upon gelatin plates—grayish-white colonies, which slowly liquefy gelatin. They grow moderately rapid. Stained with Gram's method, and have no pathogenic action.

Diplococcus Albicans Tardissimus (Bumm).—*Origin.*—In urethral pus. *Form*, like gonococci. *Properties*, immotile; do not liquefy gelatin. *Growth*, very slow at ordinary temperature, but more rapid at brood-heat. The colonies show small white points, which under low power appear brown and opaque.

Agar Stroke-culture.—Grayish-white growth, which after two months is like a skin upon the surface.

Staining.—Takes Gram's method.

Pathogenesis.—None known.

Micrococcus Subflavus (Bumm).—*Origin.*—In lochial discharges, in vagina and urethra of healthy persons.

Form.—As gonococci.

Properties.—Not motile; liquefy gelatin slowly; a yellow-brownish pigment.

Growth.—Grows slowly on all media, forming on gelatin, after two weeks, a moist yellowish surface growth.

Potato.—Small half-moon-shaped colonies which, after three weeks, become light brown in color, and covering the surface as a skin.

Staining.—Colored with Gram.

Pathogenesis.—Not acting upon the mucous membrane, but when injected in cellular connective tissue, an abscess results which contains myriads of diplococci.

The *gonococcus* is distinguished from all these similar micrococci by—

First.—Being found usually within the cell protoplasm.

Second.—Not stained with Gram's method.

Third.—Refusing to grow readily upon gelatin.

All the similar bacteria being easily cultivated.

These characteristics, taken *in toto*, form sufficient features for its ready recognition, and as it is often a serious question to decide, not so much because of the patient's health as because of his character, we should be very careful not to pronounce a verdict until we have tested the microörganism as above. When the germ is found which answers to the above description, the process can be called *specific* without a doubt.

Protective Vaccines in Pus Infections.—Wright has used the opsonic treatment in infections due to pus-forming organisms with most marked success.

Autovaccines are made by using cultures of the lesion. An agar slant is obtained, and the growth washed in normal salt solution and killed by steam. The dead bacteria in a cubic centimeter, computed according to Wright's method (which see), and known quantities of bacteria—from 250,000,000 to 500,000,000 dead cells—are injected into the patient.

The opsonic index is first estimated, and then measured every few days, giving the injection during the positive phase. If autovaccines cannot be prepared, laboratory cultures of mixed germs can be used. In boils, acne pustules, gonococcus infections, and *Bacillus coli* abscesses good results have been reported.

Bacillus of Tetanus (Nicolaiier-Kitasato).—*Origin.*—Nicolaiier found this bacillus in the pus of a wound in one who had died of tetanus, describing it in 1884.

Kitasato isolated and cultivated this germ (1889).

Form.—A very delicate, slender rod, somewhat longer than the bacillus of mouse septicemia, which is the smallest bacillus known.

When the spores form, a small swelling occurs at the end where the spore lies, giving it a drum-stick shape.

Properties.—Not very motile, though distinctly so; liquefies gelatin slowly. The cultures give rise to a foul-smelling gas.

Growth.—Develops very slowly, best at brood-heat (36° to 38° C.), and only when all oxygen is excluded—an *obligatory anaërobin*. In an atmosphere of carbon dioxid gas it cannot grow, but in hydrogen it flourishes.

Colonies on gelatin plates in an atmosphere of hydrogen. Small colonies. After four days a thick center and radiating wreath-like periphery, like the colonies of *Bacillus subtilis*.

High Stab-culture.—The gelatin having 2 per cent. glucose added and filling the tube. Along the lower portion of the needle-track, a thorn-like growth, little needle-like points shooting out from a straight line. The whole tube becomes



Fig. 103.—*Bacillus* of tetanus with spores.

clouded as the gelatin liquefies, and then the growth settles at the bottom of the tube.

Agar.—At brood-heat, on agar, the growth is quite rapid, and at the end of forty-eight hours gas-bubbles have formed and the growth nearly reached the surface.

Bouillon.—Adding glucose to the bouillon gives a medium in which an abundant growth occurs.

Cultivation from Spores.—Kitasato, by exposing a portion of suspected material to a temperature of 80° C. for one hour, killed off all the spores save those of tetanus, which were then cultivated.

Staining.—All the ordinary stains, Gram's method also, the spores being colored in the usual way.

Pathogenesis.—A small amount of the pure culture injected under the skin of experiment animals will cause, in two to three



Fig. 104.—*Bacillus tetani*: culture four days old in glucose-gelatin (Fränkel and Pfeiffer).

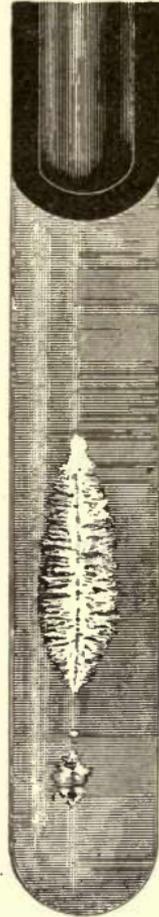


Fig. 105.—Six days' culture of bacillus of tetanus in gelatin (deep stab) (Fränkel and Pfeiffer).

days, death from true tetanus, the tetanic condition starting from the point of infection. At the autopsy nothing characteristic or abnormal is found, and the bacilli have disappeared, except near the point of entrance. This fact is explained as follows:

Several toxic products have been obtained from the cultures, and they are produced in the body, and give rise to the morbid symptoms. These have been isolated, and when injected singly, cause some of the tetanic symptoms. The virus enters the circulation, but does not remain in the tissues. The spores are very resistant to heat, drying, and chemicals.

The blood and the urine contain the toxin and are fatal to animals.

The toxin is most virulent. It acts on the end-plates of the muscles, and then on the motor nerve-cells. The incubation period is from two to fourteen days after receipt of injury.

Immunity.—Kitasato, by inoculation of sterilized cultures, has caused immunity to the effects of virulent bacilli.

An *antitoxin* obtained by Tizzoni and Cattani from the serum of animals made immune by sterilized cultures is used with curative effects in cases of tetanus in man. It is a globulin, but differs from the anthrax antitoxin, and it is found exclusively in the serum. By precipitation with alcohol and drying *in vacuo* the antitoxin is obtained in a solid state. The aqueous solution is used for injection subcutaneously or subdurally through a trephine opening. Its injection into the spinal canal by lumbar puncture has also been recommended. Antitoxin is more beneficial in chronic cases than in acute.

The dried antitoxin has been spread on the wound with some curative action.

The antitetanic serum, to be effective, must be given very early and in large doses. Its greatest use is in preventing tetanus in wounds liable to be infected. From 50 c.c. to 100 c.c. of a billion-unit serum should be given in divided doses; only serums with very high protective powers should be used.

Habitat.—The bacillus is present in garden-earth, in manure, and it has been isolated even from mortar.

The earth of special districts seems to contain the bacilli in greater quantities.

Spores of tetanus may gain access to animal serums, and if not properly destroyed, may produce tetanus during the use of these products. Great care and previous testing should

be used in the manufacture of all animal vaccines, antitoxins, etc.

Bacillus Œdematis Maligni (Koch, 1881); **Vibrion Septique** (Pasteur, 1875).—*Origin*.—In garden-earth, found also in man, in severe wounds when gangrene with edema had developed. Identical with the bacillus found in Pasteur's septicemia.

Form.—Rods somewhat smaller than the anthrax bacilli, the ends rounded very sharply. Long threads are formed. Very large spores which cause the rods to become spindle shaped.

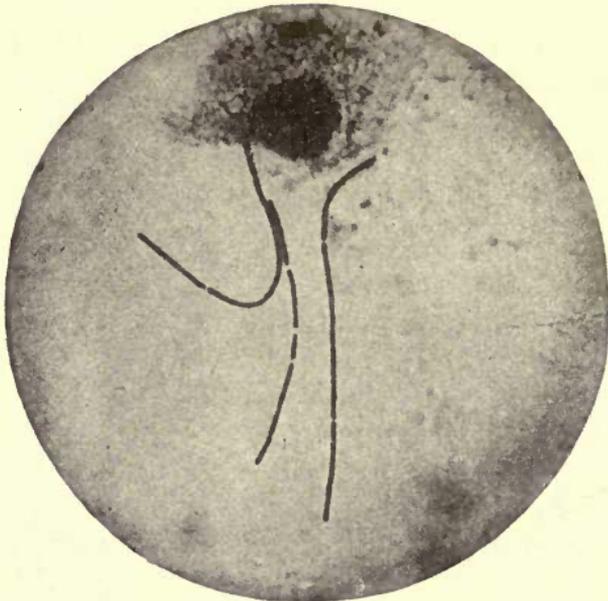


Fig. 106.—Bacillus of malignant edema, from the body-juice of a guinea-pig inoculated with garden-earth ($\times 1000$) (Fränkel and Pfeiffer).

Properties.—Very motile; liquefy gelatin; do not produce any foul gaseous products in the body.

Growth.—Grows rapidly, but only when the air is excluded, and best at brood or body heat.

Roll Cultures (After Esmarch's Method).—Small, round colonies with fluid contents, under low power, a mass of motile threads in the center, and at the edges a wreath-like border.

High Stab-culture.—With glucose gelatin, the growth at first

seen in the bottom of the tube, with a general liquefaction of the gelatin; gases develop and a somewhat unpleasant odor.



Fig. 107.—Cultures in agar of malignant edema, after twenty-four hours, at 37° C. (Fränkel and Pfeiffer).



Fig. 108.—Bacillus of malignant edema growing in glucose-gelatin (Fränkel and Pfeiffer).

Agar.—The gases develop more strongly in this medium, and the odor is more prominent.

Guinea-pig Bouillon.—In an atmosphere of hydrogen clouding of the entire culture-medium without any flocculent precipitate until third day.

Staining.—Are stained with the ordinary dyes, but Gram's method negative.

Pathogenesis.—When experiment animals, mice or guinea-pigs, are injected with a pure culture under the skin, they die in eight to fifteen hours, and the following picture presents itself at the autopsy: In guinea-pigs from the point of infection, spreading over a large area, an edema of the subcutaneous tissues and muscles, which are saturated with a clear red serous exudate, free from smell, containing great quantities of bacilli.



Fig. 109.—Smear of pus of chancroid of penis ($\times 1500$) (Davis) (photomicrograph by Mr. L. S. Brown).

The spleen is enlarged, especially in mice. The bacilli are not found in the viscera, but are present in great numbers on the surface, *i. e.*, in the serous coverings of the different organs; though when any length of time has elapsed between the death of the animal and the examination, they can be found in the inner portions of the organs, for they grow well upon the dead body. In man they have been found in rapidly spreading gangrene. They are present in the soil, in putrefactions of various kinds, and in dirty water.

Immunity.—Is produced by injection of the sterilized cultures, and also the filtered bloody serum of animals dead with the disease.

Bacillus of Soft Chancre (Ducrey-Unna, 1889).—A diplo-bacillus which is specific has been described by Ducrey as obtained from the secretion and in the depth and margins of the chancroid. Unna's bacillus is narrower and unbroken in the center.

Cultivation.—Cultivation has occurred on blood-agar, the blood being added in the proportion of one to two. *Colonies* are small, round globules.

Staining.—With borax, methylene-blue, decolorized with weak acetic acid.

Pathogenesis.—Probably a mixed infection occurs in most chancroids, especially if buboes result. The bacillus of Ducrey is not found in unopened buboes, though often contaminating the ulcerated ones.

The disease has been reproduced by inoculation of the human subject. Laboratory animals are immune.

Bacillus of Bubonic Plague (Yersin and Kitasato, 1894).—Bubonic plague or pest is an extremely infectious disease,

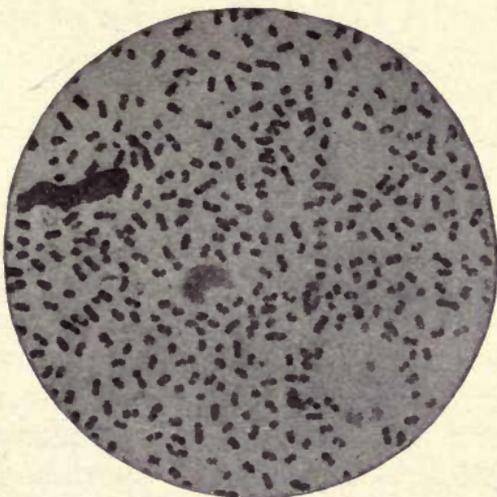


Fig. 110.—Bacillus of bubonic plague (Yersin).

more or less common in China and the East, and is believed to have its origin in man from rats and other rodents. It spreads with great rapidity, especially among those living under unsanitary conditions.

Nearly at the same time Yersin and Kitasato, working independently, discovered in the bubonic swellings and blood of affected persons a distinctive bacillus which has conformed to all the conditions necessary to make it the cause of the disease.

Origin.—In the tissues and all the body-fluids and secretions of affected individuals.

Form.—Short, thick rods with an indistinct capsule, rounded ends. Growing in chains in fluid media.

Properties.—Immotile. Stains readily. No spores. Cultivated best in oxygen, but is facultative anaërobic. Stains stronger at the ends, producing bipolar appearance. Gelatin not liquefied. *Easily destroyed by sunlight and drying.* Very resistant to cold.

Growth.—Best at 37° C.

Gelatin.—At 22° C., in twenty-four hours, white, point-like colonies on the plates, with broad and flat surface, turning gray and then brown.

Stab.—Snow-white, spreading out on the surface to the edge, and fluorescent.

Bouillon.—Granular precipitate, with clear fluid above.

Agar and Blood-serum.—Glass-like colonies like drops of dew at first, then growing larger with iridescent edges.

Potato.—At 37° C. small white mass.

No gas-formation in glucose media.

Staining readily with all basic dyes.

Pathogenesis.—After subcutaneous injection in rats death follows in forty to sixty hours, with symptoms of severe toxemia and convulsions. The point of infection shows a local edema and inflammation of the lymphatics. All the organs congested and surrounded by a bloody exudate. The characteristic bacilli in all the tissues and secretions. Nearly all the domestic animals are susceptible. Mosquitoes and pigeons, however, are immune—flies are not; fleas are a very important element in the transmission, and the rat-flea may communicate the disease to the rat from man or from the rat to man. Animals protected from the flea may live near infected animals without

danger. Direct infection by dust or other material seldom occurs.

Products.—A toxin has been obtained and immunity has been effected; the serum of immune animals has protective properties. The serum likewise shows *agglutinating* powers, as with typhoid and cholera serums.

Habitat.—Not found in water, but most likely spreads from the soil in damp and darkened areas. Rats become affected first, and then through fleas affect man and other animals. In man three forms of the disease are recognized according to the mode of infection and course of the disease—viz., bubonic, pulmonic, septicemic.

Vaccines.—The vaccines of Haffkine and Terni and Bandi have been used extensively, and with some good results.

Antitoxins.—The antitoxins of Yersin and of Lustig have been used, but without much result.

Bacillus Dysenteriae (Shiga, 1898).—The term dysentery is applied to an intestinal disease displaying more or less constancy in its clinical manifestations, but having, as is now known, a variety of causative agents. It is fairly certain that one type is the result of infection with an ameba, while non-amebic forms can probably be produced by several bacteria. Chief among those is the bacillus first described by Shiga in Japan, and since then found by Kruse in Germany, by Flexner, Strong, and Harvie in the Philippine Islands, and by Vedder and Duval in the United States. Although it is not absolutely proved that it is the cause of the disease, still the fact that it is constantly present in the feces in one type of dysentery, that such cases give a positive agglutination reaction, the production of a curative serum by the immunization of animals with pure cultures, and the results on experiment animals, leave little doubt as to the specificity of the organism.

Origin.—The dejecta of dysenteric patients.

Form.—A plump bacillus with rounded ends, resembling the typhoid and colon bacilli.

Properties.—Motility doubtful, but numerous flagella have been demonstrated. Does not form spores.

Staining.—Stains readily, negative to Gram; facultative anaërobe.

Growth.—Best at 37° C. Killed by ten minutes' exposure to 55° C.

Gelatin.—A white line of growth along puncture; superficial growth slight.

Bouillon.—Uniform clouding. Indol usually not produced; milk not coagulated.

Agar.—Resembles typhoid bacillus.

Potato.—Thin whitish layer, turning light brown.

No *gas-formation* in glucose or lactose media.

Pathogenesis.—Mice and guinea-pigs die in one or two days after intraperitoneal inoculation. Rabbits usually recover, though lesions analogous to those of human dysentery have been produced. Dogs die in five or six days, with well-marked diarrhea.

Products.—The patient's blood-serum agglutinates the bacillus in cases in which it can be cultivated from the stools. The reaction is absent from other cases. Shiga has reduced the mortality from 34.7 to 9 per cent. by means of a serum obtained from immunized horses, but in more extensive tests the antidysenteric serum proved of little value.

Habitat.—Found in the stools and in shreds of mucous membrane from the intestinal walls.

Bacillus Aërogenes Capsulatus (Welch, 1891).—*Origin*.—The intestine of man and animals, soil, sewage, and water.

Form.—A thick bacillus, 3 to 6 μ in length, frequently capsulated.

Properties.—Not motile, anaërobic, forms spores chiefly in cultures on blood-serum.

Growth.—Best at 37° C.

Gelatin.—Liquefied slowly or not at all.

Bouillon.—Forms gas.

Milk.—Coagulated and becomes acid.

Potato.—Thin, grayish-white growth with gas-production.

Forms gas in abundance on dextrose, lactose, or saccharose media.

Pathogenesis.—Is not usually pathogenic for rabbits and mice, though in guinea-pigs and birds it produces “gas phlegmons.” It is sometimes found in autopsies on human subjects, producing bubbles or cavities in the viscera (Schaumorgane), but this is probably due to postmortem migration of the germ from the intestine. It has been recovered from the blood during life, however, and is the most frequent cause of emphysematous gangrene. Various foreign observers have described organisms having similar properties, and have given them such names as



Fig. 111.—*Bacillus aërogenes capsulatus* (from photograph by Professor Simon Flexner).

Bacillus perfringens, *Bacillus enteritidis*, *Granulobacillus immobilis*, etc., but they were probably dealing with the *Bacillus aërogenes capsulatus*.

Micrococcus Melitensis (Bruce, 1887).—Malta fever, also known as Mediterranean fever, occurs in the region from which it derives its name, but has been observed in India, the Philippine Islands, and Porto Rico. Bruce cultivated a micrococcus from the spleen and proved its specificity.

Origin.—Is found most abundantly in the spleen.

Form.—Rounded or oval, $5\ \mu$ in diameter, singly, in pairs, or short chains.

Properties.—Non-motile, though flagella^s said to be present; grows slowly, best at body-temperature.

Gelatin.—Not liquefied; growth very slow.

Bouillon.—Turbid, with sediment.

Agar.—Pearly white growths.

Potato.—Slight invisible growth.

Stained by ordinary anilin dyes.

The disease may be produced in monkeys by even small amounts of pure culture. In man a chronic, remittent febrile disease is produced, with sweating and arthritis. The mortality is 2 per cent. A serum reaction can be obtained and is diagnostic.

Microorganisms have been found by various observers in measles, scarlatina, mumps, and whooping-cough, but their specificity is still in doubt.

Mode of Transmission.—Zammitt found that 50 per cent. of the goats of Malta gave the agglutination reaction to the micrococcus, and it was present in the milk in 10 per cent. Monkeys fed on the milk contracted the disease.

Preventive measures instituted in 1906 have borne out the theory that the milk of goats is the cause of Malta fever, and since the practice of importing goats from Malta has stopped, the disease has disappeared from Gibraltar. In Malta, among the troops, the fever has been greatly reduced by eliminating milk from the dietary.

Bacillus Enteritidis Sporogenes (Klein).—*Origin.*—First isolated from stools of infantile diarrhea. It is found in sewage.

Form.—Bacillus twice as long as it is broad, often containing a spore at one end. Is slightly motile and has flagella.

Growth.—Grows well under anaerobic conditions in ordinary media. Liquefies gelatin in twenty-four hours, produces acid and gas in bile-salt glucose media. In milk it separates the curd in twenty-four hours, with abundant gas-formation.

Pathogenesis.—If a small quantity of the milk culture is inoculated into a guinea-pig, the animal dies in twenty-four

hours. The skin around the point of inoculation becomes gangrenous, and foul-smelling edema, with gas-containing blebs, occurs.

Habitat.—It is a common inhabitant of the intestines of man and animals, and if found in water, is a sure indication of sewage pollution.

CHAPTER XX

PROTOZOA

PROTOZOA are unicellular animal organisms, minute as bacteria, and differing from bacteria in the methods of reproduction. Their structure and functions are more complex, although the borderland is ill defined.

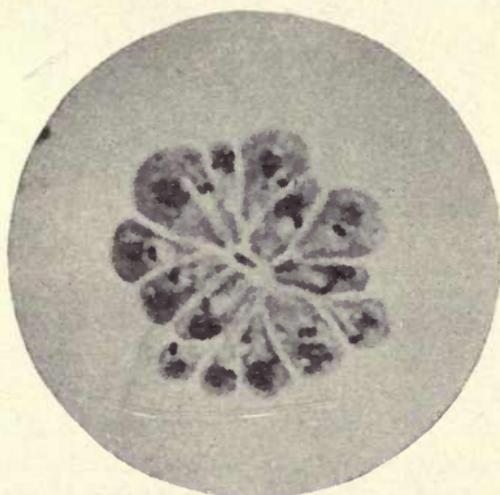


Fig. 112.—Pure culture of trypanosomes of mosquitoes—*Crithidia fasciculata*. Multiplication roset showing large and small cells. Nine-day culture (Gen. 1. $\times 1500$) (Novy, MacNeal, and Torrey).

Divisions.—There are four grand divisions of protozoa: (1) Sarcodina, containing 5500 species; (2) mastigophora,

containing 500 species; (3) infusoria, containing 700 species; (4) sporozoa, containing 300 species.

Sarcodina are chiefly marine forms, with processes changeable in shape.

Mastigophora have undulating flagella, and are known as flagellates; to this division the trypanosomata belong.

Infusoria have fine ciliary processes or numerous delicate flagella.

Sporozoa have no motile organs, and are reproduced by spores. To this division belong the coccidia of malaria and the organisms discovered by Mallory in scarlatina.

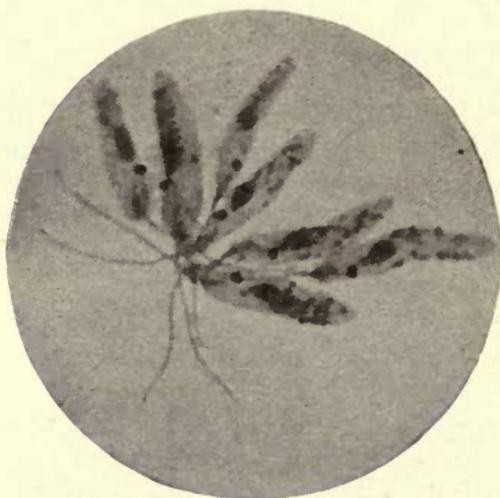


Fig. 113.—Pure culture of trypanosomes of mosquitoes—*Crithidia fasciculata*. Part of roset of elongated crithidia with flagella directed centrally (Gen. 39, $\times 1500$) (Novy, MacNeal, and Torrey).

Life-cycle.—The complete cycle of reproduction has been observed in only one of the pathogenic protozoa, namely, the protozoa of malaria.

Life Cycle of the Malarial Sporozoa.—According to its situation, the parasite exhibits two distinct phases of existence: in the human blood it passes through an *asexual* reproductive cycle, known as *schizogony*, while in the body of the mosquito it undergoes an entirely different series of *sexually* reproductive changes, called *sporogony*. It is simpler first to describe the

life history of the organism in general, pointing out the differences shown by the varieties later.

1. *The Asexual Cycle in Man*.—An infected mosquito conveys the parasites into the blood as minute hyaline bodies which enter the blood-cells. At first they are small, round, colorless bodies, exhibiting more or less active ameboid motion in the fresh blood. Sometimes, particularly in the estivo-autumnal form, a ring shape is assumed. Their size gradually increases and pigment-granules appear, while in stained specimens a nucleus containing chromatin granules is visible. As

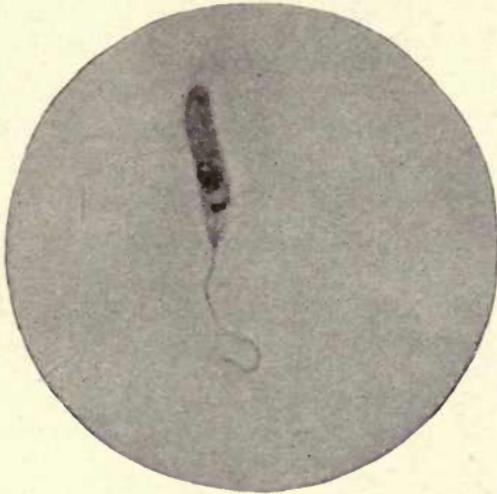


Fig. 114.—Pure culture of trypanosomes of mosquitoes—*Crithidia fasciculata*. Elongated crithidia from same preparation as preceding (Novy, MacNeal, and Torrey).

the parasite approaches maturity, the chromatin becomes scattered, and finally the protoplasm or mother-cell, known as *sporocyte*, divides into six to twenty spores, daughter-cells or *merozoites*, each containing a portion of the chromatin. The number of spores formed and their arrangement before segmentation takes place differ in the three varieties and will be noted below. The spores burst through the envelop of the red corpuscle and become free in the blood, but speedily enter fresh corpuscles and pass through the same series of changes. The febrile stage is synchronous with sporulation and liberation of the young forms.

Certain of the parasites do not, however, go on to segmentation, but, after reaching maturity, remain quiescent and form the so-called *gametes* or sexual types. In the tertian and

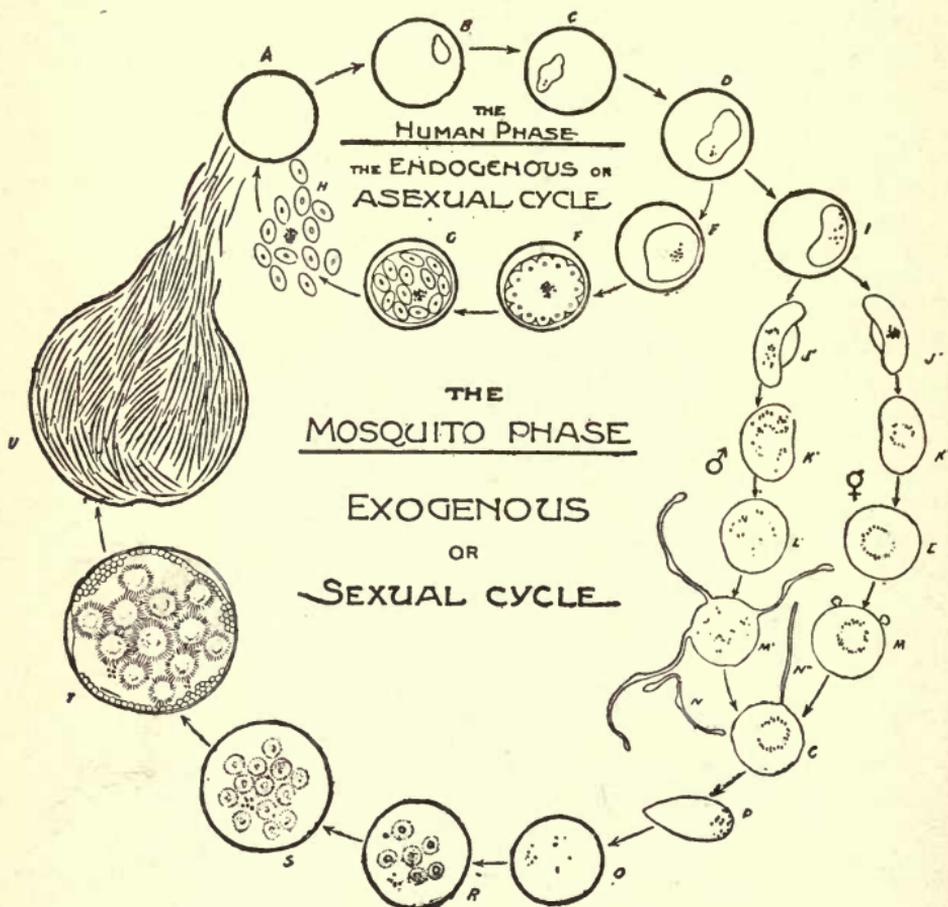


Fig. 115.—Schema showing the human and mosquito cycles of the malarial parasite: A, Normal red cell; B, C, D, E, red cells containing amebulae or myxopods; F, G, H, sporocytes; J', K', L', M', microgametocytes or male gametes; J'', K'', L'', M'', O, macrogametocytes, or female gametes; N', N'', microgametes; P, traveling vermicle; Q, young zygote; R, S, zygotomeres; T, blastophore; U, mature zygote (modified from Blanchard's diagram illustrating life-cycle of *Coccidium schubergi*) (Rees, in "Practitioner," March, 1901).

quartan varieties these are not very different from the mature organisms, but the estivo-autumnal gametes are crescentic in shape and very characteristic.

2. *The Sexual Cycle in the Mosquito.*—If, now, the blood is shed, certain of the gametes (the male forms or *microgametocytes*) extrude long protoplasmic processes containing a central core of chromatin, and which represent the male fertilizing element (*microgametes*). These become detached, and, entering a female gamete (*macrogamete*), a true sexual fertilizing process takes place. In the alimentary canal of the mosquito these fertilized cells penetrate the stomach-walls and form cysts (oöcysts) filled with a large number of filiform spores (sporozoites), which are extruded into the body cavity of the insect, and some of which reach the salivary glands, whence they are ejected when the mosquito bites. This cycle of development takes seven or eight days.

Three Forms of Malarial Protozoa.—1. *The Tertian Form.*—The adult forms are large, not very refractile, and their outline is somewhat indistinct. There is an abundance of fine pigment-granules, and the ameboid motion is vigorous. Segmenting forms divide into fifteen to twenty merozoites; the sexual forms or gametes are large. The red cell containing the organism is swollen and pale. Sporulation and, therefore, the malarial paroxysm occur every forty-eight hours.

2. *The Quartan Form.*—The organism is smaller, is more refractile, and its outline is more distinct. The pigment is coarse and situated at the periphery of the organism, while the protoplasmic motion is sluggish. Segmentation forms only six to twelve spores, and has the regular “daisy-head” appearance; the gametes are small. The red cells become dark in color, and the cycle requires seventy-two hours.

3. *Estivo-autumnal Form.*—The adult forms are found mainly in the spleen and other viscera, and do not very often occur in the peripheral blood; their outline is sharp, and they are highly refractile. The pigment is scanty and fine; the motion is active. A variable number of merozoites is formed—usually six to twelve. The gametes are characteristic, being crescentic in shape and very resistant to quinin. The red cell becomes shriveled and yellowish. The cycle usually takes forty-eight hours, though it is somewhat variable.

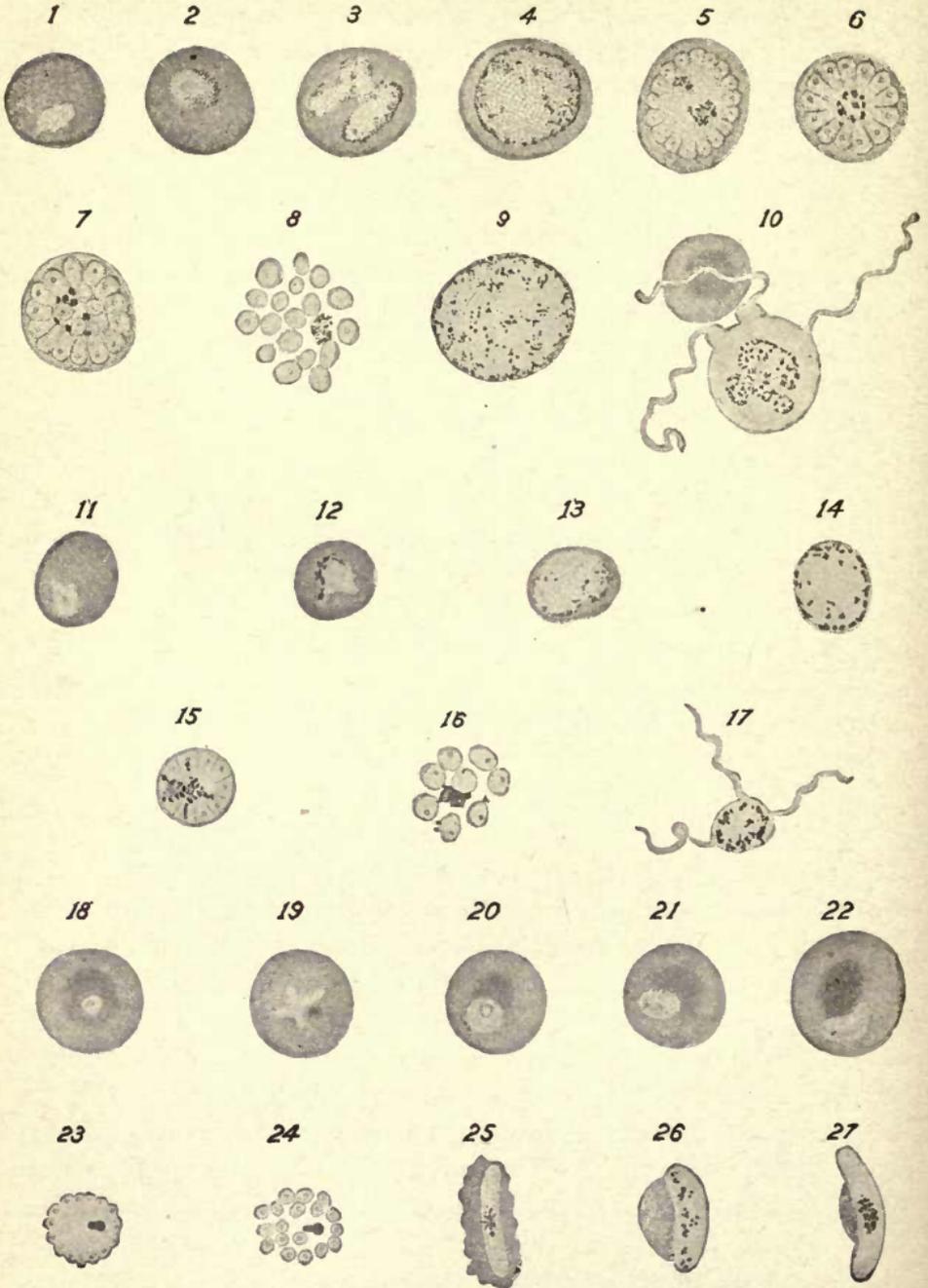


Fig. 116.

Mixed infections with the different organisms or with two or more broods of the same organism may occur, so that quotidian and irregular paroxysms may be produced.

Transmission.—Malaria is spread by means of a mosquito, the *anopheles*, in whose body the protozoön undergoes its highest development. Man is the intermediate host.

Methods of Examination for Malarial Organisms.—

1. *Fresh preparations* are made by placing a small drop of blood on a slide and a cover-glass over it, so that only a thin film is formed. A ring of vaselin is smeared over the edges of the cover-glass to prevent evaporation. This is the best method for studying flagellation and fertilization, but is less satisfactory for routine clinical work than—

2. *Stained Smears.*—These are made by spreading a drop of blood in a thin film over one slide with the edge of another, drying in the air, and staining. Many stains have been devised for the malarial organism, but the following are sufficient for ordinary use:

(1) *Marchoux's Thionin Stain.*—Add 20 c.c. of saturated solution of thionin in 50 per cent. alcohol to 100 c.c. of 2 per cent. phenol. Fix the smears and stain for fifteen to twenty seconds. The malarial organisms are stained a deep purple, strongly contrasting with the faint green of the red cells, so that they are readily recognized.

(2) *Jenner's Stain.*—This is excellent for routine work, as no preparatory fixation is required. Equal parts of a 1.2 per cent. aqueous solution of Grübler's water-soluble eosin and a 1 per cent. aqueous solution of Grübler's medicinal methylene-blue are mixed, and the resulting precipitate allowed to stand for twenty-four hours, washed, and dried. Half a gram of this is

Fig. 116.—Various forms of malarial parasites (Thayer and Hewetson): 1-10 inclusive, tertian organisms; 11-17 inclusive, quartan organisms; 18-27 inclusive, estivo-autumnal organisms.

1, Young hyaline form; 2, hyaline form with beginning pigmentation; 3, pigmented form; 4, full-grown pigmented form; 5, 6, 7, 8, segmenting forms; 9, mature pigmented form; 10, flagellate form.

11, Young hyaline form; 12, 13, pigmented forms; 14, fully developed form; 15, 16, segmenting forms; 17, flagellate form.

18, 19, 20, Ring-like and cross-like hyaline forms; 21, 22, pigmented forms; 23, 24, segmenting forms; 25, 26, 27, crescents.

dissolved in 100 c.c. of pure methyl-alcohol. The smears are dropped into this stain for one to three minutes, without previous fixation, and at once rinsed in distilled water. The malarial parasites are stained blue, the cell-bodies a reddish brown.

(3) *Wright's Chromatin Stain*.—This is the best of the chromatin stains. For its preparation, which is quite complicated, see Wright, *Journal of Medical Research*, vol. vii, 1902. It is used as follows:

1. The stain is poured over the film and allowed to remain for one minute to secure fixation.

2. Add distilled water drop by drop until a metallic scum is formed on the surface. The staining now takes place and requires two to three minutes. Wash in distilled water until a pinkish tint appears in the thin portions of the smear. The body of the malarial parasite is stained blue, and its chromatin a lilac to red color. The red cells are orange pink.

If possible, examinations for malarial organisms should always be made before quinin is administered.

Trypanosomata.—Trypanosomes are flagellate protozoa found in the blood of various animals, and causing a number of diseases, such as surra, dourine, and nagana, affecting horses and cattle, especially in tropical countries, and causing the sleeping sickness of Africa, which is very fatal for human beings.

Morphology.—A fusiform mass, containing at one end a flagellum.

In the living state these protozoa are very motile. In the stained specimen chromatin granules are found and two or more nuclei. From the smaller nucleus arises the undulatory membrane, which passes into the flagellum and assists in the wave-like motion.

In the body fluids division occurs, first of the nucleus, and then of the protoplasm.

Cultivation.—Novy and MacNeal have succeeded in cultivating these protozoa on blood-agar, and multiplication goes on rapidly, so that rosetts are formed with the flagella arranged around a common center. (See Figs. 112, 113, 114.)

Trypanosoma Lewisi (Lewis, 1878).—Found in rats; not fatal to them, though often equaling the red corpuscles in number. The infection continues for two months without producing any illness, and the animal is then immune.

Cultivated best at 20° C. and very resistant to cold. The rat is probably infected by the bite of a flea or louse. (See Fig. 117.)

Trypanosoma Brucei (Bruce, 1894) causes *nagana*, or *tsetse-fly disease*, a disease affecting horses, cattle, and dogs in certain regions of South Africa. The trypanosome of Bruce

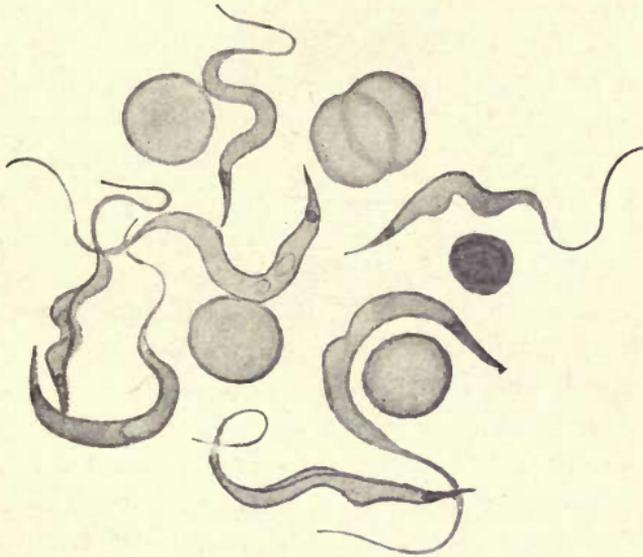


Fig. 117.—Trypanosome from blood of gray rat; stained with a 2 per cent. aqueous solution of methylene-blue (Boston).

is less motile than that of Lewis. It has been cultivated at 25° C., and is less resistant to cold. All laboratory animals subject to infection. The rat dies in ten days.

In the natural infection Bruce discovered that the tsetse-fly transmitted the disease, but that it did so by first biting some animal whose blood contained the trypanosome. The blood of infected animals contains the organism, and can, if injected, produce the disease without the agency of the fly. So far the tsetse fly alone is responsible for the spread of the infection.

Trypanosoma Ugandense Gambiense (T. Castellani, T. Hominis, T. Nèprevi).—Sleeping sickness, or human trypanosomiasis, is a disease peculiar to some parts of Africa. It is accompanied by periods of fever, anemia, and, finally, a lethargy deepening into coma and death. The disease may be rapid, and it may last with recurrences for many years. Trypanosomes identical with those found in nagana disease have been found in the blood of infected persons, and described by various observers, and given different names.

Monkeys, when inoculated with cerebrospinal fluid from affected persons, develop a similar disease, and the parasites are found in the blood.

A blood-sucking fly, known as the *Glossina palpalis*, is considered the means of infection. The fly is closely related to the *Glossina morsitans*, or tsetse fly. The sleeping sickness in man is most likely the same thing as the nagana of cattle.

Trypanosoma Evansi (Evans, 1880).—Pathogenic for all animals.

Discovered in the blood of horses suffering from surra, a disease prevalent in India and the Philippine Islands. The disease resembles nagana.

T. equiperdum and *T. Rougetii* are names given to similar organisms found in dourine, a disease affecting horses in southern France and Spain. Trypanosomes are found in fish, oysters, birds, and frogs, and many varieties have been described.

Piroplasma Bovis (P. Bigeminum) (T. H. Smith, 1893).—*Origin*.—In the blood of animals suffering from Texas cattle-fever.

Form.—A pear-shaped protozoön, found in pairs in the red cells of the blood, the smaller ends of pear in opposition; coarse ameboid movement.

Transmission.—An insect or tick (*Boöphilis bovis*) becomes infected, and by its bite infects other animals.

Other similar sporozoa have been found in animal diseases and in man in Rocky mountain fever. The *P. hominis* has been described, but not definitely determined.

Negri Bodies (Negri, 1903).—*Origin.*—Found in the nervous system of animals dying of rabies (hydrophobia).

Form.—Round and oval, hyaline bodies, with a sharp outline and containing a nucleolus. The plasma is slightly granular. They are regarded as protozoa.

Staining.—A smear from brain tissue is made on a cover-glass and fixed in methyl-alcohol for five minutes; then stained by Giemsa; stain for half-hour to three hours.

Spirillum of Relapsing Fever (Obermeier, 1873).—*Synonym.*—Spirochæta Obermeieri.

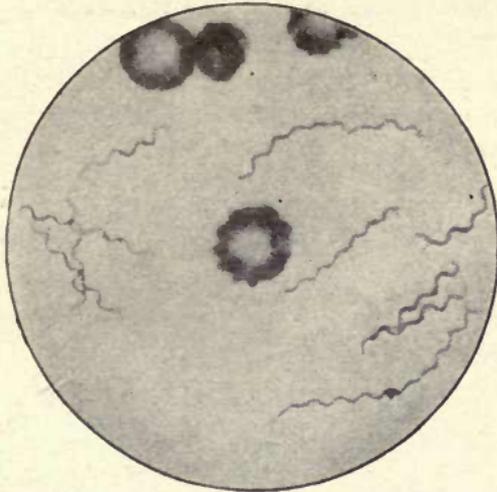


Fig. 118.—Spirochæta Obermeieri from human blood (Kolle and Wassermann).

The definite classification of this organism has not been made. Some regard it now as a protozoön, and one of a group in which numerous other spirilla belong.

Origin.—Found in the blood of recurrent fever patients, described in 1873.

Form.—Long, wavy threads (16 to 40 μ long), a true spirillum; flagella are present (Fig. 118).

Properties.—Very motile. *Has not been cultivated.*

Staining.—Ordinary anilin stains. Bismarck-brown best for tissue sections.

Pathogenesis.—Found in the organs and blood of recurrent fever. Man and monkeys inoculated with blood from one suf-

fering from this disease become attacked with the fever, and in their blood the spirillum is again found. It is found in the blood, only in the relapses (during the fever). After the attack the spirilla gather in the spleen and gradually die there. It has been found in the brain, spleen, liver, and kidneys. In the secretions it has not been discovered.

Agglutinating substances have been developed. Immunity has been produced in rats, and the serum has antitoxic properties.

Transmission.—The bed-bug retains the spirillum in its blood and is considered an important factor in spreading the disease.

African Tick Fever.—A spirochæte similar to that of relapsing fever has been observed in ticks, which conveyed a disease to monkeys similar to the above fever.

Spirochæte Pallida (Schaudinn, 1905); **Spirochæta Pallidum**; **Treponema Pallidum**.—Found in hereditary

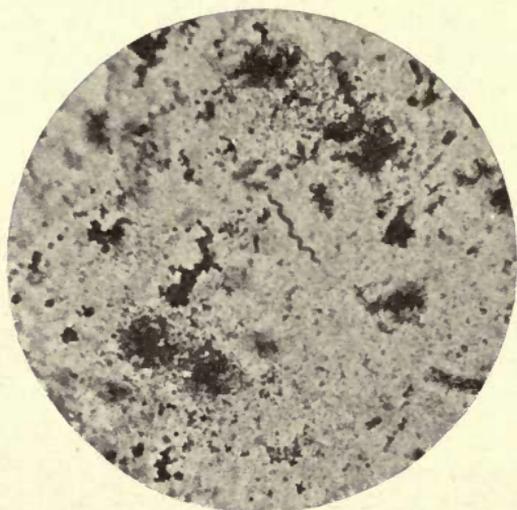


Fig. 119.—*Spirochæta pallida*. Microphotograph made by Dr. R. E. Laveson from a specimen prepared by H. Fox (Stengel).

syphilis in all organs, in chancre, and lymphatic glands, and in secondary lesions.

Form.—A minute, spiral-shaped organism, with six to eight curves, ends tapering. Actively motile in fresh specimen (Fig. 119).

Staining.—The organism requires special staining, and a number of complicated methods have been introduced by different investigators. The Giemsa stain is said to give the best results. (See Staining Fluids, p. 35.)

The slide is fixed, dried in air, hardened in absolute alcohol twenty-five minutes, stained with dilute stain (1 drop to 1 c.c. of water) for ten minutes, washed in water, and mounted.

In tissues the organism can be shown by fixing with silver nitrate after the manner of Ramon y Cajal. The tissue is—(1) Hardened in formalin for twenty-four hours (the sections should be thin); (2) washed in water for one hour; (3) alcohol, twenty-four hours; (4) 1½ per cent. silver nitrate solution in incubator at 37° C. three days; (5) washed in water twenty minutes; (6) placed in mixture of pyrogallic acid, 4 parts; formalin, 5 parts; distilled water, to make 100 parts, and kept in dark bottle for forty-eight hours; (7) washed in water and alcohol and then embedded in paraffin and sectioned. Spirochætæ black, tissues, pale yellow. Or counterstain of fuchsin can be employed.

Transmission.—Metchnikoff and Roux have inoculated anthropoid apes, producing in them the primary sore and secondary symptoms, with reproduction of the organism. There is no increase in the blood. All facts point to this organism as being the true cause of syphilis.

Classification.—It is undecided whether the spirochæte of syphilis belongs to the group of bacteria known as spirilla or to the protozoa.

Amœba Dysenteriæ.—Found in the intestinal ulcers, feces, and secondary liver abscesses in certain cases of dysentery. A non-pathogenic form, *Amœba coli*, also occurs. The *Amœba dysenteriæ* is a unicellular animal organism, measuring 25 to 35 μ in diameter, though larger and smaller forms occur. There are a nucleus and a nucleolus; the protoplasm of the cell-body is vacuolated, and often contains red blood-cells and bacteria. In fresh, warm stools active ameboid motion may be observed. The non-pathogenic form is smaller and never contains red blood-cells.

Small-pox and Vaccinia.—The exciting agent of small-pox is still unknown, but numerous bacteria and protozoön-like bodies have been described and given etiologic significance by various authors. There is some evidence in favor of Funck's belief that vaccinia is caused by a protozoön, the *Sporidium vaccinale*. Animals inoculated with this organism developed both vaccinia and variola.

Yellow Fever.—For some years it was thought that a bacillus, called *Bacillus icteroides* by Sanarelli, was the cause of yellow fever. The earlier work of Sternberg was disproved when it was shown that his bacillus, *Bacillus X*, was identical with the colon group, and Reed and Carroll found that Sanarelli's germ was an allied organism.

It is now known that a special species of mosquito, *Stegomyia fasciata*, conveys the infection and acts as a culture-medium for some unknown microörganism, possibly a protozoön, which must undergo certain changes to become virulent.

Only by the bite of a mosquito infected with the blood of a yellow-fever patient or by direct inoculation of such blood can yellow-fever be transmitted.

The experiments made so far show that the germ is destroyed by a temperature of 55° C. for ten minutes. It can pass through a Berkefeld filter, and is, therefore, extremely minute, but no one has as yet been able to find any distinctive organism in any of the blood.

CHAPTER XXI

BACTERIA PATHOGENIC FOR ANIMALS BUT NOT FOR MAN

A GREAT many bacteria have been described in diseases peculiar to the lower animals which have only slight pathogenicity for man, and only some of the more prominent varieties can be treated of here.

Bacillus of Symptomatic Anthrax (Bollinger and Feser).

—*Synonym.*—*Charbon symptomatique*, Arloing, Cornevin, and Thomas.

Origin.—This bacillus, described already in 1879, has been isolated, and by animal inoculation shown to be the cause of the “black-leg” or “quarter-evil” disease of cattle.

Form.—Large slender rods, which swell up at one end or in the middle for the spore (Fig. 120).

Properties.—They are motile, and liquefy gelatin quite rapidly.



Fig. 120.—Bacilli of symptomatic anthrax, with spores ($\times 1000$) (Fränkel and Pfeiffer).

A rancid odor is developed in the cultures.

Cultures.—The growth occurs slowly, and only in an atmosphere of hydrogen, being very easily destroyed by oxygen and carbon dioxid; grows best at 38° C.; under 15° C. no growth.

Glucose-gelatin.—In a few days little round colonies develop, which, under low power, show hairy processes around a compact center.

Stab-cultures in Full Test-tubes.—The first growth in the lower portion of the tube not very characteristic. Gases develop after a few days, and the gelatin becomes liquid.

Agar at brood temperature, in twenty-four to forty-eight hours, an abundant growth with a sour odor and abundant gas-formation.

Staining.—Ordinary methods. Gram's method is negative, but the spores can be colored by the regular double stain for spores.

Pathogenesis.—If a small amount of the culture be injected under the skin of a guinea-pig, in twenty hours a rise of temperature, pain at the site of injection, and in a few hours more death occurs. At the autopsy, the tissues are found, blackened in color and soaked with a bloody serous fluid; in the connective tissue large collections of gas, but only in the neighborhood of the point of infection. The bacilli are found in great numbers in the serum, but only appear in the viscera some time after death, when spores have developed.

The animals are usually infected through wounds on the extremities; the stalls or meadows having been soiled by the spore-containing blood of animals previously dead of the disease. "*Rauschbrand*" is the German name; "*Charbon symptomatique*," the French, from the resemblance in its symptoms to anthrax.

Immunity.—Rabbits, dogs, pigs, and fowl are immune by nature, but if the bacilli are placed in a 20 per cent. solution of lactic acid and the mixture injected, the disease develops in them. The lactic acid is supposed to destroy some of the natural resistance of the animal's cells.

When a bouillon culture is allowed to stand a few days, the bacilli therein lose their virulence, and animals are no longer affected by them; but if they are placed in 20 per cent. lactic acid and the mixture injected, their virulence returns.

Immunity is produced by the injections of these weakened cultures, and also by some of the products which have been obtained from the cultures.

Bacillus of Chicken Cholera (Pasteur).—*Synonyms*.—*Micrococcus cholera gallinarum*; *Microbe en huit*; *Bacillus avicidus*; *bacillus of fowl septicemia*.

Origin.—In 1879 Perroncito observed this cocci-like bacillus in diseases of chickens, and Pasteur, in 1880, isolated and reproduced the disease with the bacillus in question.

Form.—At first it was thought to be a micrococcus, but it has been found to be a short rod, about twice as long as it is broad, the ends slightly rounded. The center is very slightly influenced by the anilin colors, the poles easily, so that in stained specimens the bacillus looks like a dumb-bell or a figure-of-eight (Microbe en huit).

Properties.—They do not possess self-movement; do not liquefy gelatin.

Growth.—Occurs at ordinary temperature, requiring oxygen for development. It grows very slowly.

Gelatin Plates.—In the course of three days little round, white colonies, which seldom increase in size, having a rough border and very finely granulated.

Stab-cultures.—A very delicate gray line along the needle-track, which does not become much larger.

Agar Stroke Culture.—A moist, grayish-colored skin, more appreciable at brood-heat.

Potato.—At brood-heat, after several days, a very thin, transparent growth.

Staining.—Methylene-blue gives the best picture. Gram's method is not applicable. As the bacillus is easily decolorized, anilin-oil is used for dehydrating tissue sections, instead of alcohol.

Method:

Löffler's methylene-blue.....	½ hour
Alcohol.....	5 seconds
Anilin-oil.....	5 minutes
Turpentine.....	1 minute
Xylol and Canada balsam.	



Fig. 121.—Chicken cholera in blood ($\times 1000$) (Fränkel and Pfeiffer).

Pathogenesis.—Feeding the fowls with the bacilli or injecting them under the skin will cause death in from twelve to

twenty-four hours, the symptoms preceding death being those of a severe septicemia.

The bacillus is then found in the blood and viscera and the intestinal discharges, the intestines presenting a hemorrhagic inflammation.

Guinea-pigs and sheep are immune. Mice and rabbits are affected in the same manner as the fowls.

Immunity.—Pasteur, by injecting different-aged cultures into fowls, produced in them only a local inflammation, and they were then immune. But as the strength of these cultures could not be estimated, many fowls died and the healthy ones were endangered from the intestinal excretions, which is the chief manner of infection naturally, the feces becoming mixed with the food.

Bacteria of Hemorrhagic Septicemia (Hueppe).—Under this heading Hueppe has gathered a number of bacteria very similar to the bacillus of chicken cholera, differing from it and each other but very little. They have been described by various observers and found in different diseases.

The bacteria of this group color themselves strongly at the *poles*, giving rise to the dumb-bell shape. They do not take the *Gram stain*; they are without spores, and do not liquefy gelatin.

They have been placed in three general divisions:

<i>First division.</i>	{	Wild plague (Hueppe). German swine plague (Löffler, Schütz). Rabbit septicemia. Ox plague (Oresti-Armanni). Steer plague (Kitt).
------------------------	---	--

The bacteria of the first division are not motile, do not grow on potato, and are found scattered through the blood-vessels. A local reaction is uncommon.

<i>Second division.</i>	{	American swine plague (Billings). French swine plague (Cornil and Chante- messe). Frog plague (Eberth).
-------------------------	---	--

Here the bacteria are *motile*. They grow on potatoes and

are similar to the typhoid bacillus in gelatin. They form small embolic processes in the capillaries. They cause only a local disturbance in rabbits when subcutaneously injected. An acid fermentation is produced in milk.

Third division. { Hog-cholera (Salmon).
 { Swedish swine plague (Lelander).

The bacteria of this third division are very motile. The hog-cholera bacilli lie in the spleen and other organs in small masses like the typhoid bacillus.

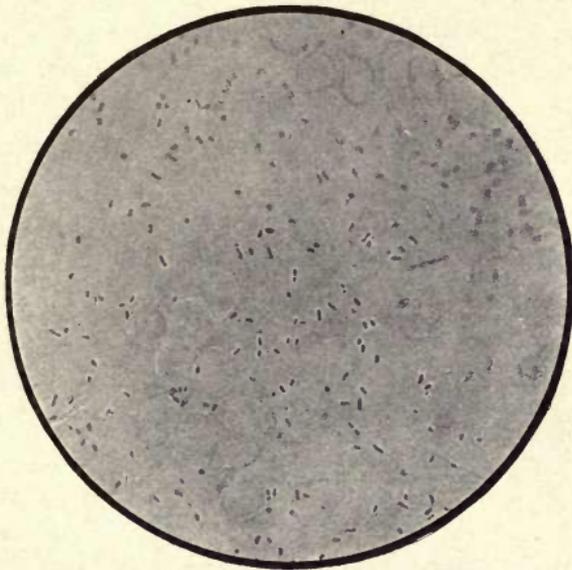


Fig. 122.—Bacillus of swine-plague (from photograph by E. A. de Schweinitz).

Rabbits die in four to eight days without any local disturbance. The growth on potato is strong.

The Swedish swine-plague bacillus occupies a position between that of *hog-cholera* and *Bacillus coli communis*.

The various swine-plague bacilli are but little active in fowls, differing thus widely from the chicken-cholera bacillus.

Bacillus of Erysipelas of Swine (Löffler, Schütz); Schweinerotlaufbacillus (German); Rouget du Porc (French).—*Origin.*—Found in the spleen of an erysipelatous swine by Löffler in 1885.

Form.—One of the smallest forms of bacilli known; very thin, seldom longer than $1\ \mu$, looking at first like little needle-like crystals. Spores have not been found.

Properties.—They are motile; do not liquefy gelatin.

Growth at ordinary temperature, very slowly, and the less oxygen, the better the growth.

Gelatin Plate.—On third day little silver-gray specks, seen best with a dark background, coalescing after a while, producing a clouding of the entire plate.

Stab-cultures.—In a few days a very light, silvery-like clouding, which gradually involves the entire gelatin; held up against a dark object, it comes plainly into view.

Staining.—All ordinary dyes and Gram's method also.

Tissue sections stained by Gram's method show the bacilli in the cells, capillaries, and arterioles in great numbers.

Pathogenesis.—Swine, mice, rabbits, and pigeons are susceptible; guinea-pigs and chickens, immune.

When swine are infected through food or by injection, a torpidity develops with diarrhea and fever, and on the belly and breast red spots occur which coalesce, but do not give rise to any pain or swelling. The animal dies from exhaustion in twenty-four to forty-eight hours. In mice the lids are glued together with pus.

At the autopsy the liver, spleen, and glands are enlarged and congested, little hemorrhages occurring in the intestinal mucous membrane and that of the stomach.

Bacilli are found in the blood and in all the viscera.

One attack, if withstood, protects against succeeding ones.

Immunity.—Has also been attained by injecting vaccines of two separate strengths.

Bacillus Murisepticus (Koch); Mouse Septicemia.—

Origin.—Found in the body of a mouse which had died from injection of putrid blood, and described by Koch in 1878.

Form.—Differs in no particular from the bacillus of swine erysipelas, excepting that it is a very little shorter, making it the *smallest* known bacillus. Spores have been found, the cultures exactly similar to those of swine erysipelas.

The pathologic actions are also similar. Field-mice are immune, whereas for house and white mice the bacillus is fatal in two to three days.

Micrococcus of Mal de Pis (Nocard).—Gangrenous mastitis of sheep.

Origin.—In the milk and serum of a sheep sick with the “mal de pis.”



Fig. 123.—Bacillus of mouse septicemia, from the blood of a mouse ($\times 1000$) (Fränkel and Pfeiffer).

Form.—Very small cocci seldom in chains.

Properties.—Immotile; liquefying gelatin.

Growth.—Growth occurs best between 20° and 37° C., is very rapid, and irrespective of oxygen.

Plates of Gelatin.—White round colonies, some on the surface and some in the deeper strata, with low power, appearing brown surrounded by a transparent areola.

Stab-culture.—Very profuse along the needle-track, in the form of a cone after two days, the colonies having gathered at the apex.

Potato.—A dirty gray, not very abundant, layer, somewhat viscid.

Staining.—With ordinary methods; also Gram's method.

Pathogenesis.—If a pure culture is injected into the mammary gland of sheep, a "*mal de pis*" is produced which causes the death of the animal in twenty-four to forty-eight hours. The breast is found edematous, likewise the thighs and perineum; the mammæ very much enlarged, and at the nipples a blue-violet coloration. The spleen is small and black; other animals are less susceptible. In rabbits abscesses at the point of infection, but no general affection.

Bacillus Alvei (Cheshire and Cheyne); Bacillus melitophtharus (Cohn).—*Origin.*—In foul-brood of bees.

Form.—Slender rods, with round and conical pointed ends; very large oval spores, the rod becoming spindle-shaped when they appear.

Properties.—Motile, liquefying gelatin rapidly.

Growth.—Grows best between 20° C. and 37° C., very slowly; aërobic.

Gelatin Plates.—Small grooves are slowly formed, which unite so as to form a circle or pear-shaped growth, from which linear grooves again start.

Stab-culture.—Grows first on surface, then gradually along the needle-track, long processes shooting out from the same, clouding the gelatin. Later, air-bubbles form like the cholera culture, and in two weeks the whole gelatin liquefies.

Staining.—Do not take anilin dyes very well. Gram's method is, however, applicable.

Pathogenesis.—If a pure culture is spread over the honey-comb containing bee larvæ, or if bees are fed upon infected material, foul-brood disease will occur. Mice, if injected, die in a few hours. Edema around the point of infection, and many bacilli contained in the edematous fluid, otherwise no changes.

Bacterium Termo (Cohn).—This was a name given to a form of microörganism found in decomposing albuminous material, and was supposed to be one specific germ. Hauser, in 1885, found three different distinct bacilli which he grouped under the common name of proteus, which have the putrefying properties ascribed to *Bacillus termo*.

Proteus Vulgaris.—*Origin.*—In putrid animal matter, in the feces, and in water.

Form.—Small rods, slightly curved, of varying lengths, often in twisted chains, having long cilia or flagella.

Properties.—Very motile, and very soon liquefying gelatin; forms hydrogen sulphid gas; causes putrefaction in meat.

Growth.—Growth very rapid, best at 24° C.; is facultative aërobic.

Gelatin Plates.—Yellowish-brown, irregular colonies, with prolongations in every direction, forming all sorts of figures; an impression preparation shows these spider-leg processes to consist of bacilli in regular order.

Stab-culture.—The gelatin soon liquid, a gray layer on the surface, but the chief part of the culture in small crumbs at the bottom.

Pathogenesis.—Rabbits and guinea-pigs injected subcutaneously die quickly; a form of toxemia, hemorrhagic condition of lungs and intestines present. When *neurin* is injected previously, the animals do not die. This ptomain is supposed to be generated by the *Proteus vulgaris*.

Proteus Mirabilis (Hauser).—Differs from *Proteus vulgaris* in that the gelatin is less rapidly liquefied. Found also in putrid material.

Proteus Zenkeri (Hauser).—Does not liquefy gelatin; otherwise similar to the other two.

CHAPTER XXII

YEASTS AND MOLDS

IN works on bacteria these true fungi, *yeasts* and *molds*, are usually considered. They are so closely related to bacteria, and so often contaminate the culture-media, and are so similar in many respects, that a description is almost a necessity.

But there are several thousand varieties, and we cannot attempt to describe even all the more important ones. It will answer our purpose to detail a few of the more common kinds, and give the principal features of the different orders.

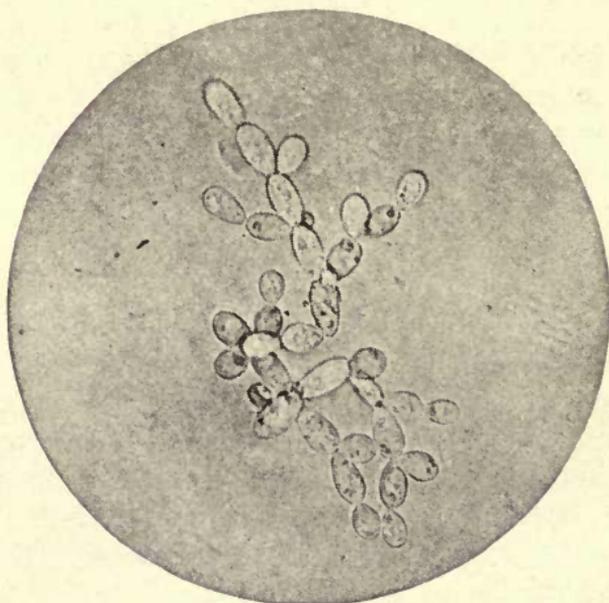


Fig. 124.—Yeast-cells ($\times 500$) (Fränkel and Pfeiffer).

Saccharomycetes or **yeasts** increase through budding; the spores are attached to the mother-cell like a tuber on a potato.

Yeasts are the cause of alcoholic fermentation in the saccharoses. A description of the most common ones will suffice.

Saccharomyces Cerevisiæ (**Torula Cerevisiæ**).—This is the ordinary beer-yeast.

Form.—Round and oval cells; a thin membrane inclosing a granular mass, in which usually can be seen three or four irregular-shaped spores. When these become full grown, they pass through the cell-wall and form a *daughter-cell*. Sometimes long chains are produced by the attached daughter-cells.

Growth.—They can be cultivated as bacteria are in bouillon, but grow best in beer.

There are several varieties of beer-yeast, each one giving a characteristic taste to the beer. Brewers, by paying special attention to the nutrient media, cultivate yeasts which give to their beers individual flavors.

Mixed yeast gives rise to a poor quality of beer.

Saccharomyces Rosaceus; S. Niger; S. Albicans.—These yeasts are found in the air; and instead of producing alcoholic fermentation, they give rise to a pigment in the culture-media. They grow upon gelatin, which they do not liquefy.

Saccharomyces Mycoderma.—This yeast forms a mold-like growth, or skin, on the surface of fermented liquids, but does not cause any fermentation itself. It forms the common "mold" on wine, preserves, and "sour-kROUT."

Pathogenic Yeasts.—A number of workers have interested themselves in experiments with yeasts in their relation to disease; and under the name of *blastomycetes*, Sanfelice has grouped yeasts that produce tumors resembling epitheliomata; and he has tried to prove that the so-called animal parasites found in malignant growths, and variously known as coccidia and sporozoa, are yeasts. These are, however, protozoa.

Oidium.—A form which seems to be the bridge between the yeast and the molds is the *oidium*. Sometimes it resembles the yeasts, sometimes the molds, and often both forms are found in the same culture. Several are pathogenic for man.

Oidium Lactis.—*Origin.*—In sour milk and butter.

Form.—The branches or hyphæ break up into short, rod-like spores. No sporangium, as in molds.

Growth.—In milk it appears as a white mold.

Artificially cultured on gelatin plates, or milk-gelatin plates,

it forms satin-like, star-shaped colonies, which slowly liquefy. Under the microscope the form of the fungus is well seen.

Agar Stroke Culture.—The little stars, very nicely seen at first; then the culture becomes covered with them, causing a smeared layer to appear over the whole surface, with a sour odor.

Properties.—The milk is not changed in any special way. It is not pathogenic for man or animals. It is found when the milk begins to sour.

Oidium Albicans (Soor; Thrush Fungus, Langenbeck, 1839).—*Origin.*—Mucous membrane of the mouth, especially of infants.

Form.—Taken from the surface of the culture, a form like yeasts; but in the deeper layers, mycelia with hyphæ occur.

Growth.—Not liquefying; snow-white colonies on gelatin plates.

Stab-culture.—Radiating yellow or white processes spring from the line made by the needle, those near the surface having oval ends.

Potatoes.—The yeast form develops as thick white colonies.

Bread-mash.—Snow-white veil over the surface.

Pathogenesis.—In man the parasitic thrush, or “white mouth,” is caused by this fungus. In the white patches the spores and filaments of this microbe can be found. Rabbits receiving an intravenous injection perish in twenty-four to forty-eight hours, the viscera being filled with mycelia.

Blastomycosis or Oidium Mycosis.—A skin disease described, in 1894, by Gilchrist, and since then by other writers, is due to a fungus which resembles yeast, and which has been called a blastomyces; but Ophuls and Ricketts term it an oïdium, and the former calls the parasite *Oidium coccidioides*.

Form.—The fungus increases by budding, but in culture-media it may resemble a mold or oïdium.

Pathogenesis.—Small abscesses form in wart-like lesions, which extend over large areas of the skin, becoming later on systemic and invading lungs and kidneys; abscesses and nodules form in these organs.

True Molds.—Flügge has made five distinct divisions of molds. It will, however, serve our purpose to classify those to be described under three headings: *Penicillium*, *Mucor*, and *Aspergillus*.

Penicillium Glaucum.—*Origin.*—The most widely distributed of all molds, found wherever molds can exist.

Form.—From the mycelium, hyphæ spring which divide into basidia (branches), from which tiny filaments arise (sterigmata), arranged like a brush or tuft. On each sterigma a little bead

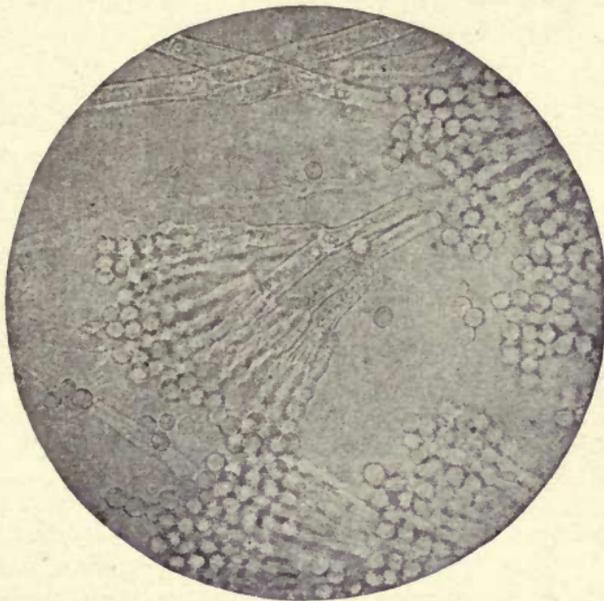


Fig. 125.—*Penicillium glaucum* ($\times 500$) (Fränkel and Pfeiffer).

or conidium forms, which is the spore. In this particular fungus the spores in mass appear green.

Growth.—It develops only at ordinary temperatures, forming thick, grayish-green molds on bread-mash. At first these appear white, but as soon as the spores form, the green predominates. Gelatin is liquefied by it.

Mucor Mucedo.—Next to the *Penicillium glaucum*, this is the most common mold. Found in horse-dung, in nuts and apples, in bread and potatoes, as a white mold.

Form.—The mycelium sends out several branches, on one of

which a pointed stem is formed which enlarges to form a globular head, a spore-bulb, or *sporangium*. The spore-bulb is partitioned off into cells in which large oval spores lie. When the spores are ripe, a cap forms around the bulb, the walls break down, and the wind scatters the spores, leaving the cap or "*columella*" behind.

Growth.—Takes place at higher temperatures on acid media. It is not pathogenic.

Achorion Schönleinii. **Trichophyton Tonsurans.** **Microsporon Furfur.**—These three forms are similar to each other in nearly every particular, and resemble in some

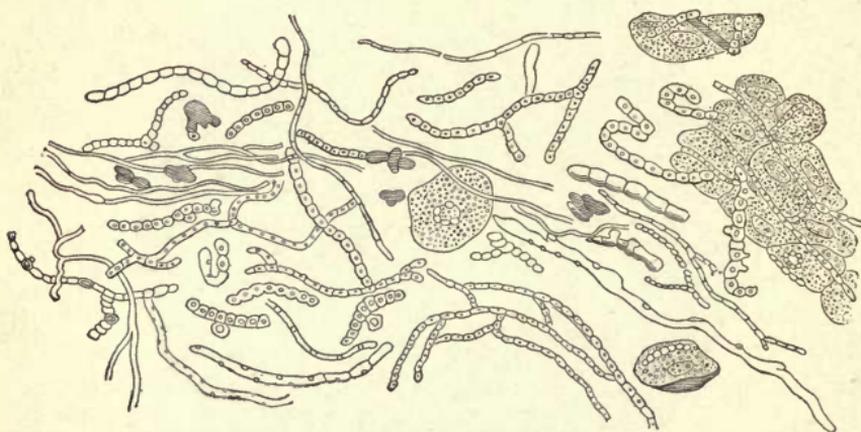


Fig. 126.—*Achorion Schönleinii* (after Kaposi).

respects the *Oidium lactis*, in other ways, the mucors. The first one, *Achorion Schönleinii*, was discovered by Schönlein in 1839, in *favus*, and is now known as the direct cause of this skin disease.

Origin.—Found in the scaly crusts of *favus*.

Form.—Similar to *Oidium lactis*.

Growth.—Is very sparse. On gelatin round white masses inclosed by a zone of liquefied gelatin.

In milk it is destroyed.

Pathogenesis.—Causes *favus* in man.

Trichophyton Tonsurans.—Found, in 1854, by Bazin, in *tinea*.

Form.—Similar to the achorion or favus fungus.

Growth.—Somewhat more rapid than the favus, and the gelatin quickly liquefied. Old cultures are of an orange-yellow color. Colonies have a star-shaped form.

Pathogenesis.—Herpes tonsurans and the various tineæ are produced by this fungus.

Microsporon Furfur.—Found in tinea versicolor, almost identical with the above, forms dry yellow spots, usually on the chest, in persons suffering from wasting diseases.



Fig. 127.—*Aspergillus fumigatus* ($\times 500$) (Fränkel and Pfeiffer).

Aspergillus Glaucus.—*Origin.*—In saccharine fruits.

Form.—The hypha has formed upon its further end a bulb, from which pear-shaped sterigmata arise and bear upon their ends the conidia or spores.

Growth.—Best upon fruit-juices. *Non-pathogenic.* The mold is green. *Aspergillus flavus* has the tufts and spores of a yellow color.

Aspergillus Fumigatus.—Is pathogenic for rabbits when injected into them. At the autopsy their viscera are found filled with the mold.

Examination of Yeasts and Molds.—Yeasts and molds are best examined in the unstained condition. A small portion of the colony rubbed up with a mixture of alcohol and a few drops of liquor ammonia; of this, a little is brought upon the glass slide, covered with a drop of glycerin; and the cover-glass pressed upon it. If the preparation is to be saved, the cover-glass is secured by ringing around the edges with varnish or cement. *Yeasts* take *methylene-blue* stain very well.

Cladotriches and Streptotriches.—The streptotrich and cladotrich groups are classed with the higher bacteria, but

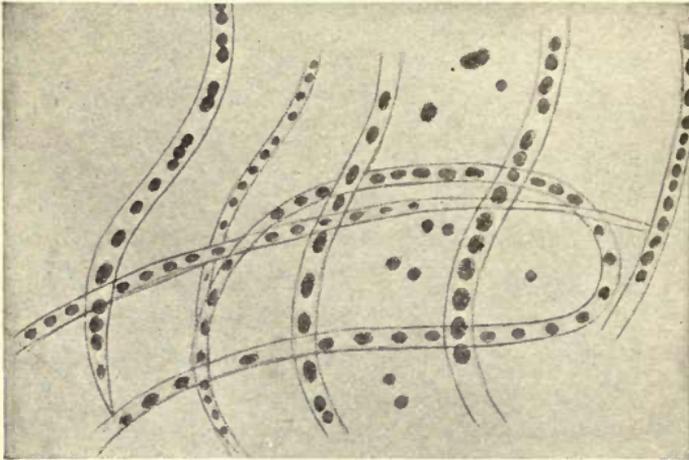


Fig. 128.—*Cladotrich dichomata* from well-water (one-twelfth oil-immersion. Fuchsin stain) (author's specimen).

their exact status is still undetermined. They may be considered as representing the transition from the bacteria to the lower fungi.

Streptotrich or Cladotrich Actinomyces (Ray-fungus).—Actinomycosis is a disease caused in man and cattle by this organism, which is commonly found in grain, particularly barley. It is probable that several varieties of the parasite can produce the characteristic lesions. It has been discovered in all countries and in various organs of the body, although its place of election is about the lower jaw, where it tends to form hard, ulcerating abscesses, affecting other organs secondarily.

Form.—In the granular masses of an abscess cylindrical fila-

ments are matted together, and radiating outward from this zone are club-shaped branches, as the petals of an aster. In the center of the granule are numerous cocci-like bodies, and some of the ovoid or club-shaped hyphæ lie detached from the clusters. Through cultivation it was found that the ovules give rise to filaments, and they then form the ovules again.

Cultivation.—At 38° C. on glycerin-agar in a period of one to two weeks, pointed scales about the size of a millet-seed, center

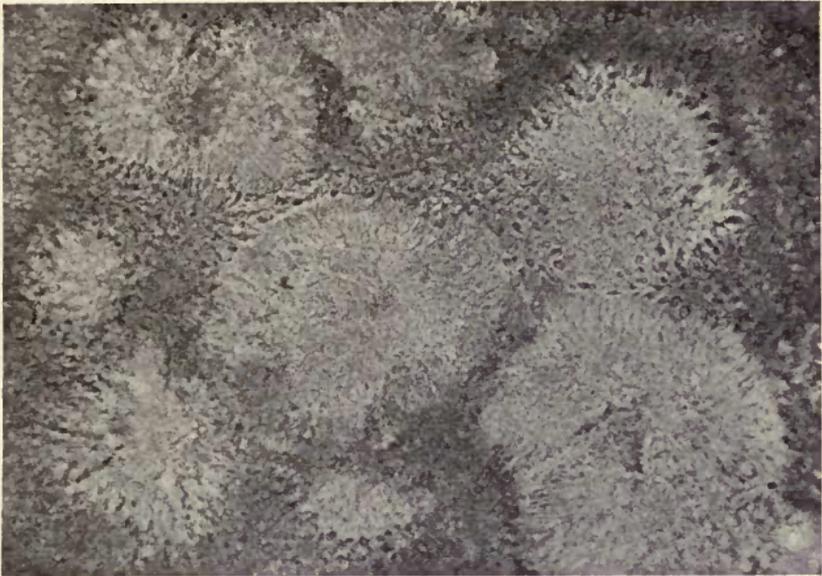


Fig. 129.—Actinomyces granule crushed beneath a cover-glass, showing radial striations in the hyaline masses. Preparation not stained; low magnifying power (Wright and Brown).

dry and prominent, margins hyaline, composed only of filaments, short and long, massed together, but no clubbed forms.

By some the *clubs* are considered the spore organs; by others, they are thought to be encapsulated or thickened filaments.

Pathogenesis.—When a portion of the growth obtained in eggs was injected into the abdominal cavity of a rabbit, actinomycotic processes developed upon the peritoneum.

It usually gains access to the living body through a wound in the gum or some caries of the teeth. A new growth is formed, ulceration being first set up.

The new tissue, composed of round-cells, then undergoes softening, purulent collections form, and the normal structure is destroyed.

The usual seat is in the maxillary bones, but the fungus has been found in the lungs, tonsils, intestines, and various other organs in man and cattle.

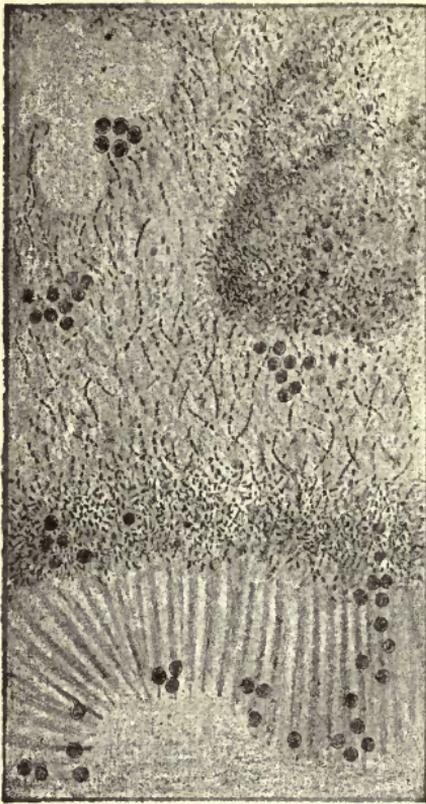


Fig. 130.—*Streptothrix Maduræ* in a section of diseased tissue (Vincent).

Examination.—Well seen in the unstained condition. From the pus or scraping a small portion is taken and squeezed upon the glass slide; if calcareous matter is present, a drop of nitric acid will dissolve the same.

Glycerin will preserve the preparation.

Staining.—Cover-glass specimens stained best with Gram's method. Tissue sections should be stained as follows:

Ziehl's carbol-fuchsin, ten minutes. Rinse in water.

Concentrated alcoholic solution of picric acid, five minutes. Rinse in water.

Alcohol, 50 per cent., fifteen minutes. Alcohol absolute, clove-oil, balsam.

The rays stained red, the tissue yellow.

***Streptothrix Maduræ* (Vincent).**—*Origin.*—Found in the disease known as Madura foot, or mycetoma, an ulceration affecting the feet, especially of individuals living in the tropics. Two varieties, the *pale* and the *black*, have been described.

Form.—Branched filaments resembling the actinomyces streptothrix in the mycelia. Spores are seen.

Cultivation.—In liquid media containing vegetable infusions

growth occurs best. Temperature of 37° C. most suited. The colonies near the surface become colored red.

Agar.—Glazed colonies, at first colorless, then rose-colored, about the size of a pea, with the central part umbilicated and pale. Gradually the rose color fades.

Acid Potato.—A slow and meager growth.

Pathogenesis.—Only local reaction has been caused by inoculation in animals. In man the disease usually follows a slight injury and attacks the leg or foot, slowly forming a nodular growth, which in the course of months or a year begins to soften and ulcerate, and with the seropus are discharged numerous little granules, some black, some pink, containing mycelia. The limb becomes much deformed, the tissue vascularized, and the degenerated area filled with the streptothrix filaments.

Staining.—The organism itself stained with ordinary stains. Gram's method for the tissue.

Streptothrix Farcinica (Nocard); Bovine Farcin du Bœuf.—*Origin*.—A disease affecting cattle and giving rise to tubercle-like lesions in the lungs, liver, and spleen. Common in France.

Form.—Small interwoven mass of threads arranged in tufts found in the centers of the tubercles.

Culture.—At body-temperature in various media.

Bouillon.—Colorless masses, irregular in size and shape.

Agar and Gelatin.—Small, rounded, opaque colonies, thicker at the periphery.

Potato.—Rapid growth of pale-yellow, dry scales, consisting of many spores.

Pathogenesis.—Pure cultures introduced into the peritoneum of guinea-pigs give rise in nine to twenty days to tubercle-like lesions. Subcutaneous injections cause abscesses with secondary involvement of the lymphatics, ending in recovery. Dogs, horses, and rabbits are immune.

Staining.—Wright's double stain for tissues; also Gram's.

CHAPTER XXIII

EXAMINATION OF AIR, SOIL, AND WATER

Air.—Many germs are constantly found in the atmosphere about us. Bacteria unaided do not rise into the air and fly about; they usually become mixed with small particles of dirt or dust and are moved with the wind. The more dust, the more bacteria, and, therefore, the air in summer contains a greater number than the air in winter, and all the other differences can be attributed to the greater or less quantity of dust and wind.

Methods of Examination.—The simplest method is to expose a glass or dish covered with gelatin in a dust-laden atmosphere or in the place to be examined. In the course of twenty-four to forty-eight hours colonies will be seen formed wherever a germ has fallen. But this method will not give any accurate results in regard to the number of bacteria in a given space; for such a purpose somewhat more complicated methods are needed, so that a certain amount of air can come in contact with the culture-media at a certain regulated rate of speed.

Hesse's Method.—This is the oldest and most useful of the various methods in vogue.

A glass cylinder, 70 cm. long and 3.5 cm. in diameter, is covered at one end by two rubber caps, the inner one having a hole in its center 10 mm. in diameter; and at the end *B*, a rubber cork fits in the cylinder; through this cork a glass tube 10 mm. in diameter passes, which is plugged at both ends with cotton. The cylinder and fittings are first washed in alcohol and sublimate and then placed for one hour in the steam-chamber.

Removing the cork of the cylinder, 50 c.c. of sterile gelatin in a fluid condition are introduced and rolled out on the sides of the tube, after the manner of Esmarch, leaving a somewhat thicker coating along the under side of the cylinder. The *aëroscope*, as the cylinder and its fittings are called, is placed

upon an ordinary photographer's tripod and the glass tube, which passes through the rubber cork, connected with an *aspirator*, the cotton having first been removed from its outer end. The aspirator consists of two ordinary wash-bottles connected with each other by a rubber tube, *C*. They are attached to the tripod with a small hook, one above the other, the upper one half filled with water and slightly tilted.

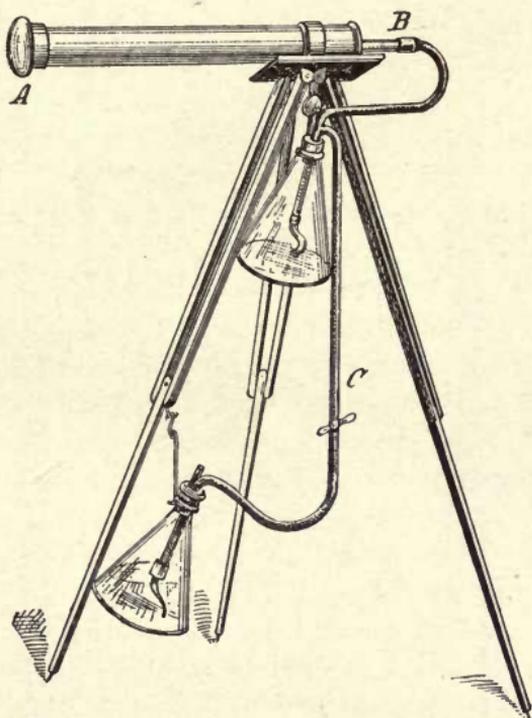


Fig. 131.—Hesse's apparatus for collecting bacteria from the air (McFarland).

When the apparatus is wanted, the outer rubber cap at the end *A* of the *aëroscope* is removed; the air can then pass through the small hole in the other cap, and the germs fall upon the gelatin in the tube, the cotton in the small glass tube at the other end preventing the germs from getting out. The aspirator is set in use by tilting the upper bottle so that the water flows into the lower; this creates suction and draws the air through the *aëroscope*.

The amount entering is estimated by the capacity of the wash-bottle, the rate at which it enters depending upon the rate of the flow of water, which can be regulated.

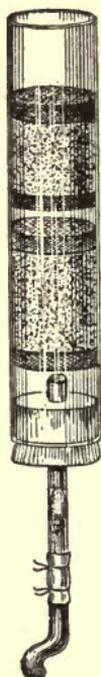


Fig. 132.—Petri's sand-filter for air-examination (McFarland).

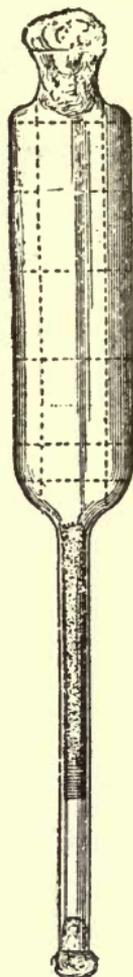


Fig. 133.—Sedgwick's expanded tube for air-examination (McFarland).

Hesse advises for rooms and closed spaces 1 to 5 liters, at the rate of two minutes a liter, and for open spaces, 10 to 20 liters, at four minutes a liter. Plate cultures can be made from the colonies, which develop in eight to ten days in the cylinder.

Petri's Method.—The air is pumped or sucked through sand-filters, and the sand then mixed with gelatin.

Sand is sterilized by heating to redness, and while still warm placed in test-tubes, which are then plugged. (Sand which has been passed through a sieve with meshes 0.25 mm. wide is the kind required.) A glass tube 9 cm. long is provided with two portions of sand, each 3 cm. long and $\frac{1}{2}$ cm. apart, little plates of brass gauze keeping the portions in position.

The tube and its contents are now sterilized in a hot-air oven at 150° C., the ends having first been plugged with cotton.

One end of the tube is then fitted with a rubber cork through which passes a glass tube, which is connected with an aspirator (a hand-pump with a known capacity).

If a 100 liters of air pass through the tube in fifteen minutes, the germs should all be arrested in the first sand-filter. And when the filters are removed and thoroughly mixed with gelatin, each filter for itself, there should be no colonies developed from the second filter, *i. e.*, the one nearest the aspirator.

Sedgwick-Tucker Method.—A special form of tube is used, called an *aërobioscope*. It consists of a neck 2.5 cm. in length, an expanded portion 15 cm. long, and a long narrow tube of 15 cm. After sterilization the tube is partly filled with granulated sugar, which is the filtering material. By means of a vacuum gage and an air-pump, or ordinary aspirating bottles, the volume of air passing through the apparatus can be determined. After the air has been passed through, the sugar is gently shaken from the narrow tube into the expanded portion, and 20 c.c. of liquefied gelatin is poured in. The sugar dissolves, and the mixture is then rolled on the inner side of the glass as an Esmarch tube. This part of the apparatus is divided into squares to make the counting of colonies easy. The *aërobioscope* is very highly recommended.

Varieties Found in Air.—The only *pathogenic bacteria* found with any constancy are the *Staphylococcus aureus* and *citreus*; but any bacterium can, through accident, be lifted into the atmosphere, and in certain places may be always found—the *Bacillus tuberculosis*, for example, in rooms where many consumptives are living.

Typhoid fever, influenza, pneumonia, and diphtheria may be

conveyed through the air by those who are infected by coughing and expectorating.

Non-pathogenic.—The micrococci predominate. *Sarcinae*, yeasts, and molds constantly contaminate cultures.

In the ordinary habitations the average number of germs to the liter of air does not exceed five.

Around water-closets, where one would imagine a great number to exist, owing to the undisturbed condition of the air, but few will be found.

Examination of Water.—The bacteriologic examination of water is to-day of as much importance as the chemical analysis, and must go hand in hand with it.

A water containing thousands of germs to the cubic centimeter is far less dangerous than one containing but two germs, if one of these two be a typhoid bacillus. It is not the number that proves dangerous, it is the kind.

If a natural water contains more than 500 germs to the cubic centimeter, it were well to examine its source.

Bacteriology performs the greatest service in testing the devices which are intended to render water fit for drinking.

As a diagnostic aid, the examination is of but little use. An epidemic of typhoid fever occurs, the water is suspected, an examination is undertaken; but the days of incubation and the days passed before the water is analyzed have given the typhoid germs, if any had been present, ample time to disappear, since in water that contains other bacteria they live but a few days only. Again, the water tested one day may be entirely free and the next day contain a great number, and before the typhoid germ can be proved to be present in that particular water the epidemic may be past. Human sewage contamination is determined by finding the colon bacillus, and if this is found in the course of an epidemic of typhoid the water containing it may well be suspected as being the cause.

Purity of Waters.—The purest water we have is the natural spring-water—water that has slowly filtered its way through various layers of gravel and sand and comes finally clear and sparkling from the ground. It is without germs; but let such

a water stand walled up in cisterns or wells, it becomes as surface water, open to all sorts of impurities, and the bacterial nature of it changes every moment.

Artesian or Driven Well.—The *driven well* will secure to a certain extent a pure water. It is the only form of well or cistern that will insure this, since the water does not become stagnant in it; but it may connect with an outhouse, the soil being very loose, allowing the products of germs of refuse water to find their way into the well. The casing may not be water-tight and surface water can be sucked in.

Filtered Water.—Dangerous as surface water is, the greater quantity used is such, the inhabitants of larger towns and cities using chiefly the rivers and other large waters which course near them for drinking purposes. A purification or filtration can, in a certain measure, render these waters harmless.

Filtration is carried on on a large scale in the water-works of cities and towns, and bacteriologic examination is here of great service to determine if a water, which has been filtered and may have a very clear appearance, and give no harmful chemical reaction, is entirely free, or nearly so, from germs; in other words, if the filter is a germ-filter or not; daily tests are necessary in order to insure safety.

Charcoal sponge and asbestos, the materials formerly in use, are objectionable because germs readily develop on them and clog them, so that they require frequent renewal. In very large filters, sand and gravel give the best results; the number of germs in a cubic centimeter is reduced to forty or fifty and kept at that number. This is a very pure water for a city water, though, as we stated before, not a safe one, for among those forty germs very dangerous ones may be found. It is then necessary for the users to refilter the water, before drinking it, through a material which will not allow any germs to pass, or, in the presence of an epidemic, to boil all water used for drinking purposes.

Pasteur-Chamberland Filter.—This very perfect filter consists of a piece of polished porcelain in the form of a

cylinder closed at one end and pointed at the other. It is placed in another cylinder of glass or rubber, and the pointed portion connected with a bottle containing the water, or directly with the faucet of the water-pipe. The water courses through the porcelain very slowly and comes out entirely free from germs; pipe-clay, bisque, infusorial earth, and kaolin are also perfect filters. The only disadvantage is the long time it takes for the water to pass through. Pressure is used to accelerate the passage in the form of an aspirator or air-pump.

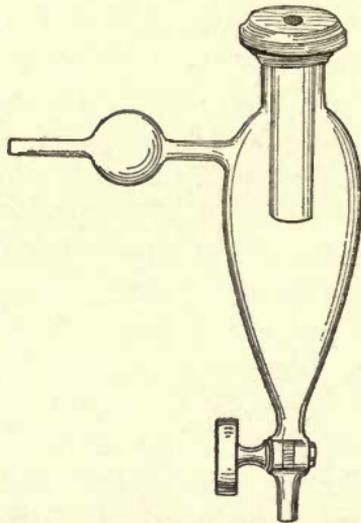


Fig. 134.—Flask fitted with porcelain bougie for filtering large quantities of fluid.

The force of the hydrant water is also sufficient to produce a steady, small stream.

These porcelain cylinders can easily be sterilized and the pores washed out.

All the cylinders or bougies are not germ proof, so that they must be tested, and most of them must be cleaned every fourth day, or they will allow germs to pass through.

Boiling as a Means of Purifying.—When such a filter cannot be obtained, the only alternative is to boil all the water to be used for drinking; and this should especially be done in times of typhoid and cholera epidemics.

Varieties Found.—The usual kinds found are non-pathogenic, but, as is well known, typhoid, cholera, and dysentery are principally spread through drinking-water, and many other germs may find their way into the water. Some of the common varieties give rise to fluorescence or produce pigment.

Eisenberg gives 100 different varieties as ordinarily found. Other intestinal diseases also are supposed to be water borne, and the presence of the *Bacillus coli communis* means sewage contamination. Ice supplies require the same supervision as water supplies, for many bacteria, like the typhoid bacillus, retain their vitality for weeks after freezing.

Methods of Examination.—Since the germs rapidly multiply in stagnant water, an examination must not be delayed longer than possible after the water has been collected. Every precaution must be taken in the way of cleanliness to prevent contamination; sterilized flasks with glass stoppers, pipets, and plugs must be at hand, and the gelatin tubes be inoculated on the spot. If this cannot be done, the sample should be packed in ice until it arrives at the laboratory. If it is necessary to send the sample by rail, the bottle containing the sample should be wrapped in sterilized cloth or the neck covered with tin-foil and the bottles placed in tin boxes (about 4 ounces—100 c.c.—is sufficient for bacterial analysis), and then packed in cotton or paper to prevent breakage and surrounded by plenty of ice until it reaches its destination. As soon as it arrives at the laboratory the sample is placed in a sterilized glass flask, and the flask then closed with a sterile cotton plug. A sterilized pipet is then dipped into the flask, and 1 c.c. of the water withdrawn and added to a tube of gelatin, the gelatin being in a fluid condition. To a second tube, $\frac{1}{2}$ c.c. is added. The tubes are then shaken so as to thoroughly mix the water with the gelatin, and then poured upon wide glass plates or porous covered Petri dishes, one plate for each tube; the plates are then placed in the moist chamber and in two or three days examined. A temperature of 18° to 20° C. is best. Many water-bacteria are hindered by higher degrees of heat. If the germs are equally divided, there should be one-half the number

on one plate that there is on the other; thus the $\frac{1}{2}$ c.c. serves as control.

Water that is very rich in germs requires dilution with sterilized water fifty to one hundred times. Fewer colonies will be found on agar than on gelatin, even at the same temperature.

Special Media and Preparation.—Sodium chlorid must not be used in the preparation of media for water analysis. The reaction of most culture-media should be +1 per cent. to phenolphthalein.

Sugar broths should be *neutral*, and must be sterilized carefully in steam and not overheated, to prevent inversion of the sugar.

Examination for Bacillus Coli and Sewage Bacteria.—Instead of examining for typhoid bacilli, sewage contamination is best indicated by the presence of the colon group of organisms.

The committee of the Public Health Association recommends the following procedure:

Two Methods.—*Method a.*—Preparation of an agar plate with a known volume of water, using lactose litmus-agar and incubating at 40° C. *Bacillus coli* will show its presence by red colonies (acid fermentation of the sugar); further testing is then needed to fully identify.

Method b.—Cultivation, at 40° C., of a measured quantity of water in a fermentation tube containing a sugar broth. If gas appears, a portion of the liquid is plated as in method *a*.

Additional Details.—If in twenty-four hours no red colonies appear in the agar-lactose litmus Petri dishes, *Bacillus coli* is considered absent, providing the sample was a polluted one, so that the bacilli, if present, would be in a concentrated form. Only 1 or 2 c.c. of water can be used, because the ordinary water-bacteria spread rapidly and contaminate the other bacteria.

If acid-forming colonies are found, five or six are fished for subcultures on slanted agar, in fermentation tubes, milk, gelatin, peptone solution, and nitrate broth.

If the water is not strongly contaminated, an underground

water, for instance, or a mountain stream, the better way is to inoculate two or three lactose or dextrose bouillon fermentation tubes and place in an incubator at 40° C. Note the presence of gas, if any, at the end of twelve, twenty-four, thirty-six, and forty-eight hours. If no gas forms, sewage bacteria are absent.

If gas forms, plate at once a portion of the sediment as above on lactose litmus-agar. Test the other fermentation tubes for acidity, and the nature of the gas, whether any, and how much is absorbed by a 2 per cent. solution of sodium hydroxid. *Bacillus coli* should produce between 30 and 70 per cent. of gas, of which about one-third is CO₂ and is absorbed by the alkali; the remainder is hydrogen. The other broth culture can be tested for the presence or absence of unfermented sugar, for the color reaction of *Rivas*.

Quantitative Tests.—The number of acid colonies in 1 c.c. and in 5 c.c. of water is taken as a measure of pollution, together with the total number of colonies of all bacteria present. Thus in 1 c.c. on the gelatin plate at 20° C. there may be fifty colonies; on the agar plate at 37° C. ten colonies, five of which were acid-formers, or presumably *Bacillus coli*.

To count the colonies which develop upon the plates, a special apparatus has been designed, known as—

Wolfhügel's Apparatus.—A glass plate divided into squares, each a centimeter large, and some of these subdivided. This plate is placed above the gelatin plate with the colonies, and the number in several quadrants taken, a lens being used to see the smaller ones.

The Petri saucers can be used instead of plates, and an apparatus on the Wolfhügel plan can be obtained to count the colonies. It is best to count all the colonies on the plate or dish.

Diagnostic Points of Colon Bacillus.—*Microscopic*.—Non-spore-bearing motile bacillus.

Gelatin.—Non-liquefactive.

Dextrose Broth.—Fifty per cent. gas; one-third absorbed, CO₂; two-thirds, hydrogen.

Milk (litmus) coagulated in forty-eight hours and rendered acid; litmus colored red.

Peptone Solution.—Production of Indol.—(A peptone solution tube is inoculated with the culture and kept together with a control four days at 37° C. Then 2 drops of concentrated sulphuric acid and 1 centimeter of a 0.01 per cent. solution of sodium nitrite are added. The appearance of a pink color at the end of thirty minutes denotes the presence of indol.)

Presumptive Test.—If a water from a well or spring produces gas in the sugar broth and forms acid colonies on litmus-lactose agar, the presumption is strong that there is sewage contamination. If gas-production continues in a series of samples carefully collected for several days or weeks, there can be no doubt of a contamination, and especially if the well or spring is protected from surface water. Algæ which grow in service pipes, reservoirs, and deep wells may give rise to non-acid gas fermentation, but all well-water that, without further testing, forms acid colonies on litmus-agar lactose plates and ferments sugar broth, is open to suspicion, and if there is evidence of the presence of typhoid fever or diarrheal diseases, the water should be boiled and subjected to careful analysis daily. There may be serious contamination and the chemical tests show no appreciable increase in the chlorids.

Bacterial Treatment of Sewage.—Where sewage is to be rendered innocuous before being allowed to flow into streams, the process of nature has been imitated by the construction of septic tanks in which the sewage remains excluded from the air and subject to the action of the anaërobic bacteria present in the sewage. The organic nitrogen is reduced, and compounds of hydrogen and sulphur are formed. The effluent is then filtered through coke-beds, where the aërobic bacteria assist in further purification.

Sewage is also treated by sedimentation with alum and filtration of the effluent over larger beds, or allowed to percolate through the soil, which is thereby enriched and utilized for agriculture.

The Examination of the Soil.—The upper layers of the soil contain a great many bacteria, but because of the difficulty in analyzing the same, the results are neither accurate nor constant. The principal trouble lies in the mixing of the earth with the nutrient medium; little particles of ground will cling to the walls of the tube, or be embedded in the gelatin, and may contain within them myriads of bacteria. As with water, the soil must be examined immediately or very soon after it is collected, the bacteria rapidly multiplying in it.

When the deeper layers are to be examined, some precautions must be taken to avoid contamination with the other portions of the soil. One method, very laborious and not often practical, is to dig a hole near the spot to be examined and take the earth from the sides of this excavation.

Fränkel's Borer.—Fränkel has devised a small apparatus in the form of a borer, which contains near its lower end a small cavity, which can be closed up by turning the handle, or opened by turning in the opposite direction.

It is introduced with the cavity closed, and when it is at the desired depth, the handle is turned, the earth enters the cavity, the handle again turned, incloses it completely, and the borer is then withdrawn.

The earth can then be mixed with the gelatin in a tube, and this gelatin then rolled on the walls of the tube after the manner of Esmarch, or it can be poured upon a glass plate, and the colonies developed so.

Another method is to wash the earth with sterilized water, and the water then mixed with the gelatin, as many of the germs are taken up by the water.

The roll-cultures of Esmarch give the best results, many of the varieties usually found being anaërobic.

Animals inoculated with the soil around Berlin die almost always of *malignant edema*, and with that of some other towns invariably of *tetanus*. Many of the germs found are nitrogen formers and play a great rôle in the economy of the soil.

Nitriifying organisms are found in the superficial layers of the earth. Organic matters found in sewage and in the fecal

evacuations of animals form the basis for their activity, whereby nitrates, ammonias, and nitric acid result. The nitrogen necessary for the growing plant is thus produced. The nitromonas of Winogradsky belongs to this group. The soil tends to destroy ordinary disease-bacteria in a short time, but spores may remain dormant for a number of years as the spores of anthrax.

The Bacteria of Milk and Other Foods.—Milk as secreted is sterile, but at every step in its passage from the cow to the consumer it is liable to contamination. Even the lower portion of the teat is a source of infection, owing to the presence of stagnated milk from the former milking, and, as milk ready for consumption usually contains thousands to millions of bacteria to the cubic centimeter, sterilization or pasteurization and supervision of the dairies should always be carried out on milk used for infant feeding.

A standard milk should be free from pus and should not contain more than 10,000 bacteria to the cubic centimeter.

Leukocytes are normally found in milk, and only when their number exceeds one million and pyogenic organisms are also present can pus be said to exist. Pasteurization of unclean milk is sometimes more dangerous as a food than untreated milk, because, by preventing the action of lactic-acid formers, other bacteria are permitted to develop and produce pathogenic toxins.

Pure Milk.—A pure milk is one that is obtained from a healthy cow, well groomed, in a clean room, by a healthy, clean person, in clean cans or bottles, and transported to the consumer in as short time as possible without further handling, keeping the container in the mean time at a low temperature and protected from the air. Such treatment is safer than any form of *sterilization*.

Foods as a Source of Infection.—Foods eaten after little or no cooking, such as fruits, salads, and the like, and also oysters, are possible sources of bacterial diseases, and the not infrequent so-called ptomain poisoning observed after the

consumption of ice-cream, sausage, canned meats, etc., is the result of the action of bacteria or their products.

Oysters and fish from sewage-polluted waters have produced typhoid. Vegetables grown in manured ground or sprinkled with polluted water may be a possible source of disease. The practise of exposing meats and other food to street dust and flies is no doubt responsible for some disease.

CHAPTER XXIV

BACTERIOLOGIC EXAMINATION OF THE ORGANS AND CAVITIES OF THE HUMAN BODY

THE body, on account of its constant contact with the surrounding air, is necessarily exposed to infection, and we would be likely to find on the skin and in the oral, anal, and nasal cavities the varieties of microorganisms commonly around us. Through the water and food the body is also contaminated; but some organisms by predilection inhabit the mouth, intestine, and other cavities, and form there a flora distinctly their own.

The Skin.—The majority of microorganisms met with on the skin are non-pathogenic, although underneath the nails and in the hair, pus-forming microorganisms often occur, producing sometimes serious abscesses on other parts of the body.

In the sweat-glands and the sebaceous glands various organisms have been found. The *Staphylococcus epidermidis albus* of Welch is present normally.

In foul-smelling perspiration of the feet Rosenbach found *Saprogenes* No. II, which is pathogenic for rabbits.

Micrococcus cereus albus and *flavus*, *Diplococcus liquefaciens albus* and *flavus*, *Staphylococcus pyogenes aureus*, and *Streptococcus pyogenes* are found underneath the nails.

In eczema, *Diplococcus albicans tardus*, *Diplococcus citreus*

liquefaciens, *Diplococcus flavus liquefaciens*, and *Ascobacillus citreus*.

In colored sweat, *Micrococcus hæmatoides*, *Bacillus pyocyaneus*.

A diplococcus is found in acute pemphigus.

The lepra bacillus, the tubercle bacillus in lupus, and the typhoid bacillus in the eruption of typhoid fever are a few of the specific germs found on the skin.

The Conjunctiva.—The micrococcus of trachoma, the Koch-Weeks bacillus, considered to be the specific cause of acute catarrhal conjunctivitis, or “pink eye,” and the *Bacillus xerosis*, are special germs found on the conjunctiva; the other varieties of air- and water-organisms, and those usually present on the skin, are also found. Löffler’s bacillus and the pneumococcus have been found in some forms of conjunctivitis. The Koch-Weeks bacillus is the most contagious.

A special diplobacillus, known as the bacillus of Morax-Axenfeld, produces a stubborn form of conjunctivitis.

The Mouth.—The mouth is a favorite seat for the development of bacteria. The alkaline saliva, the particles of food left in the teeth, the decayed teeth themselves, all furnish suitable soil for their growth.

Quite a number of germs have been isolated and their properties partly studied. Many have some connection with the production of caries of the teeth, as Miller has well shown in his careful studies. The *Leptothrix buccalis*, found in nearly all mouths, is a long chain or filamentous bacillus which stains blue with iodine. It was formerly considered the cause of tartar on the teeth.

The *Spirillum sputigenum*, *Spirochæta dentium*, *Micrococcus gingivæ pyogenes*, *Bacillus dentalis viridans*, *Bacillus pulpæ pyogenes*, micrococcus of sputum septicemia, and *Micrococcus salivarius septicus* are a few of the germs cultivated by Miller and Biondi from the mouth. Besides these, the pneumobacteria, diphtheria bacillus, and tubercle bacillus are often met with, the first two in the mouths of healthy persons. The expired air in quiet respiration is free from bacteria, but in

coughing, sneezing, etc., large numbers of organisms are violently ejected and the atmosphere about tubercular patients is always saturated with tubercle bacilli.

Ear.—In the middle ear of newborn infants no pathogenic organisms were found, but quite a number of non-pathogenic ones. In affections of the ear the pneumobacillus and the *Staphylococcus pyogenes* are most frequent.

When the streptococcus is present in acute suppurations, there is great danger of mastoiditis. In chronic otitis the gas-forming bacteria, as well as *Bacillus pyocyaneus*, is often found.

Nasal Cavity.—The nasal secretion, containing as it does dead cells and being alkaline in reaction, forms a good soil for the growth of germs.

Diplococcus coryzæ, *Micrococcus nasalis*, *Bacillus foetidus ozænæ*, *Bacillus striatus albus et flavus*, *Bacillus capsulatus mucosus*, and *Vibrio nasalis* are some of the organisms described by various observers.

Stomach and Intestine.—The secretion of the stomach is in its normal state not a favorable soil for the development of bacteria, yet some germs resist the action of the gastric juice and flourish in it. When the acids of the stomach are diminished in quantity or absent altogether, the conditions for the growth of bacteria are more favorable. The alimentary canal of the newborn infant is sterile, but in a few hours microorganisms begin to appear.

Some gastric bacteria normally present are *Sarcina ventriculi*, *Bacterium lactis aërogenes*, *Bacillus subtilis*, *Bacillus amylobacter*, *Bacillus megaterium*.

The intestinal organisms are more numerous, and the mucous lining of the intestines and the secretions there present are favorable to germ-growth.

Bacillus geniculatus, Boas considers a sign of carcinoma of the stomach, and is always present, he claims, when the contents contain lactic acid.

Some investigators consider digestion dependent on microbic activity, but experiments with animals have shown that life

and digestion can proceed in a perfectly sterile condition. Food and air sterilized will not develop bacteria in the feces.

In the feces of the young a great many bacteria have been found that are supposed to stand in close relation with the intestinal disorders common to nurslings. The majority of bacteria usually present in the intestines are non-pathogenic. The following varieties may be met with in the feces: *Micrococcus aërogenes*, *Bacillus subtilis*, *Bacillus butyricus*, *Bacillus putrificus coli*, *Bacillus lactis aërogenes*, *Bacillus coli commune*, *Bacillus subtiliformis*, and the bacteria of cholera, dysentery, and typhoid, besides many yeast-cells.

Genito-urinary Passages.—In vaginal secretion Bumm has been able to find a number of organisms, some of which closely resemble the gonococcus; thus, there is the *Diplococcus subflavus*, *Micrococcus lacteus faviformis*, *Diplococcus albicans amplus*, and the vaginal bacillus.

In the urethra of healthy persons bacteria are sometimes found, usually having entered from the air.

In the normal secretions around the prepuce a bacillus called the smegma bacillus has been discovered, and it is considered identical with the so-called syphilis bacillus of Lustgarten.

In urethral pus a number of diplococci other than the gonococci have been isolated.

From the urine itself a great number of bacteria have been obtained, but mostly derived from the air, finding in the urine a suitable soil.

Microorganisms of the Blood.—Many of the bacteria described in the body of this book are found in the blood of the animal they infect; thus, anthrax bacilli are always found in the blood, but tubercle bacilli seldom, if ever, enter this secretion.

When animals are subcutaneously injected with pneumococci they are found in large quantities in the blood. The diseases of a hemorrhagic nature affecting fowls and swine usually show the presence of bacteria in the vascular system.

Bacteria may be recovered from the blood in all forms of

septic infection, such as general sepsis, malignant endocarditis, puerperal sepsis, and typhoid fever.

Method of Examination.—A drop of blood is spread on a cover-glass and stained with the ordinary dyes; but in order to eliminate the coloring-matter of the red corpuscles and bring the stained bacteria more prominently into view, Gunther recommends that the blood, after drying and fixing, should be rinsed in a dilute solution of acetic acid (1 to 5 per cent.). The hemoglobin is thereby extracted, and the corpuscles appear then only as faint outlines.

Instead of “fixing” by heat, Canon employs alcohol for five minutes, especially in staining for influenza bacilli, which have been detected in the blood.

This method, however, requires the presence of enormous numbers of bacteria in order to succeed, and the plan commonly employed consists in making “blood cultures.” As large a quantity of blood as possible—never less than 10 c.c.—is taken from a superficial vein, the median basilic, for example, by means of a sterile antitoxin syringe, a small incision being made through the skin over the vein in order to avoid skin infection. The blood so obtained is immediately transferred to culture-tubes, which are then studied in the customary manner.

CHAPTER XXV

ANTISEPTICS AND ANTISEPSIS

A *germicide* is an agent capable of destroying bacterial life.

An *antiseptic* solution or substance is one that can inhibit or prevent the growth of bacteria without necessarily destroying them.

A *disinfectant* must be germicidal.

A *deodorant* may have no germicidal or antiseptic properties.

Preservatives are substances which prevent fermentation, but they are not always germicides.

In considering the value of a germicide, the strength in which it acts is the main consideration. Some very weak chemicals will inhibit and destroy the growth of bacteria if used in sufficiently concentrated solutions. Some bacteria will die in an acid media; others are destroyed by too much alkali. Some bacteria are very readily destroyed in pure cultures, but are resistant to a considerable degree in the body tissues. Again, a germicide may be ideal in laboratory experiments, but wholly impractical at the clinic.

Germicides are tested by action in various dilutions or in gaseous form on threads impregnated with virulent and spore-forming organisms. The length of time is noted that it takes to destroy anthrax bacilli or pyogenic organisms.

The infected material is subjected to the solution and then inoculated on media and compared with control, or tested for virulence on animals. Spore-forming organisms are very resistant to the most potent agents.

Heat is perhaps the best general *germicide*. For all articles that can be subjected to boiling or the direct flame there is no safer agent.

Superheated steam, or steam under pressure, is now in general use in sterilizing surgical dressings and instruments, and requires less time than ordinary *steam*.

The salts of metals of high atomic weights come next in order. Bichlorid of mercury and cyanid of mercury are the most powerful of chemical germicides, but in the human body they can be used in dilute solutions only, and in contact with highly albuminous solutions, insoluble and inert albuminates are liable to form, lessening the germicidal value. A 1:200 solution combined with an acid will destroy the spores of anthrax in one hour, but much weaker solutions will destroy the anthrax bacilli in the blood, and for all practical purposes a 1:2000 solution is sufficient, destroying bacterial life in a few minutes.

Phenol in 5 per cent. solution will destroy most of the bacteria in less than five minutes.

Formaldehyd, in gaseous form or in a liquid spray, is a very efficient germicide, and from the fact that it is not destructive to fabrics or paper has come into general use as a disinfectant. In combination with potassium permanganate or in suitable generators it is employed in houses after infectious diseases. It has no effect on insects, and where it is necessary to destroy these, other agents, known as *insecticides*, must be used in connection with the gas. The gas should be in a moist state—from 6 to 16 ounces for an ordinary room are needed; the room should be made as air-tight as possible, and the gas evolved as speedily as possible.

In the permanganate method 8 ounces (by weight) of potassium permanganate crystals are placed in a large tin vessel ten times the capacity of the disinfectant used. One pint of formaldehyd solution is quickly poured over the crystals. Formaldehyd gas is thereby generated at once. This will produce enough gas for disinfection of 1000 cubic feet.

Sulphur dioxid, or sulphurous acid gas, is a germicide and insecticide, and is much used in disinfecting after yellow fever and malaria. It is obtained by burning sulphur in a pan over water, and about 1 pound to a room is necessary.

Alcohol, iodin, chlorin, potassium permanganate, hydrogen dioxid, the salts of silver, lead, and zinc, salicylic acid, boric acid, anilin dyes (methyl-violet and methylene-blue), naphthalin, and creosols are a few of the substances in use as antiseptics and germicides in surgery. Their power varies with the strength of the solution and all have limitations.

In surgical operations more dependence is placed to-day on securing and maintaining a germ-free or a septic condition than on the attempt to destroy germ life by chemicals. The irritation of antiseptics in some instances prevents the natural defense forces (phagocytes) from acting, and in abdominal operations, where no pus has been encountered, the blood-serum or normal salt solution is alone used.

Sterilization of Hands, etc.—It has been shown by elaborate experiments that the skin, the hair, and clothing harbor many bacteria, some of a pathogenic nature. The surgeon who is anxious to secure good results should carefully attend to his toilet; the use of operating gowns, rubber gloves, operating shoes, face guards is now universal. The toilet of the hands of the surgeon is as important as that of the field of operation, but with the use of rubber gloves the painstaking directions as to the employment of a half-dozen or more cleansing agents and germicides are no longer followed.

Soap is an efficient germicide, the lye being in most cases powerful enough to prevent the growth of germs.

Filtration.—In the laboratory and on a larger scale in the management of water-works, filtration is a method of sterilization, acting as it does by mechanically separating bacteria from a solution.

CHIEF CHARACTERISTICS

PART I.—

Name.	Genus.	Biology.	Product.
ACETI.	Bacillus.	Short motile rods in zooglœa; ærobic.	Ferment.
ACIDI LACTICI.	Bacillus.	Short, immotile rods; ærobic.
ACIDI LACTICI.	Bacillus.	Short, immotile rods.
ACTINOBACTER.	Bacillus.	Immotile rods with capsule; facul. anærob.
AEROGENES.	Bacillus.	Small motile rods, single and in pairs; very resistant.
AEROPHILUS.	Bacillus.	Slender rods in threads; immotile; oval spores; ærobic.
AGILIS.	Micrococcus.	Mobile diplococci with fine flagella.	Red pigment.
ALBA.	Beggiatoa.	Cocci and spirals with sulphur.
ALBA.	Sarcina.	Small cocci in packets.	White pigment.
ALBICANS AMPLUS.	Micrococcus.	Large cocci and diplococci.
ALBICANS TARDIS-IMUS.	Micrococcus.	Diplococci colored by Gram.
ALBICANS TARDUS.	Micrococcus.	Diplococci not motile.
ALLII.	Bacillus.	Very small rods.	Alkaloid pigment
AMYLIFERUM.	Spirillum.	Rigid spirilla with spores; turns blue with iodine.

OF THE PRINCIPAL BACTERIA.

NON-PATHOGENIC BACTERIA.

Culture Characters.	Actions.	Habitat.	Discoverer.
Not liquefy; membranous growth.	Produces acetic-acid fermentation.	Air.	Kützing.
Not liquefy; small white points porcelain-like; slow.	Lactic-acid fermentation; precipitates caseine.	Air; sour milk.	Pasteur.
Growth faster than above; appearance same.	Alcohol is formed after the lactic-acid fermentation.	Sour milk.	Grotenfeldt.
.	Causes fermentation with gas and alcohol.	Air.	Duclaux.
Rapid growth; round, concentrically-arranged colonies; not liquefy.	Digestive tract.	Miller.
Liquefy rapidly; small yellow-gray colonies.	Old cultures.	Liborius.
Slowly liquefying, forming a cone with rose-red color.	Drinking-water.	Ali Cohens.
.	Sulphur springs.	Vauch.
Slow growth in small white colonies.	Air and water.	
Slowly liquefy; gray colonies; growth fairly rapid.	Is colored by Gram's method.	Vaginal secretion.	Bumm.
Small white points, not liquefying; very slow growth.	Urethral pus.	Bumm.
Grows slowly on surface, the boundary raised; twice as large as above.	Skin in eczema.	Unna, Tommasoli.
Bright green pellicle on agar.	Decomposes albumin.	Green slime of onions.	Griffiths.
.	Water.	VanTiegham.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
AMYLOBACTER.	Bacillus.	See <i>Butyricum</i> , with wh	ich it is identical.
AQUATILIS.	Micrococcus.	Very small cocci in irregular groups.
ARACHNOIDEA.	Beggiatoa	Very thick filaments containing sulphur; motile.
ARBORESCENS.	Bacillus.	Thin rods, with rounded ends in threads, and singly; immotile.	Yellow pigment.
ATTENUATUM.	Spirillum.	Threads with narrowed ends.
AURANTIACA.	Sarcina.	Small cocci in pairs and tetrads; strongly aerobic.	Orange-yellow pigment.
AURANTIACUS.	Bacillus.	Motile, short thick rods, often in long threads.	Orange-yellow pigment.
AURANTIACUS.	Micrococcus.	Oval cocci in pairs and singly; immotile.	Orange-yellow pigment in water, alcohol, and ether; insoluble.
AUREA.	Sarcina.	Cocci in packets.	Golden-colored pigment; soluble in alcohol.
AUREUS.	Bacillus.	Straight motile rods lying parallel.	Golden-yellow pigment.
BALTICUS.	Bacillus.	Short rod.	Phosphorescence.
BIENSTOCKII.	Bacillus.	See <i>Putrificus</i> , coll.	
BILLROTHII.	Micrococcus (ascococcus).	Groups of cocci surrounded with capsule; zoogloea aerobic.
BRUNNEUS.	Bacillus.	Motile rods.	Brown pigment.
BUTYRIC-ACID FERMENTATION.	Bacillus.	Large, slender motile rods in pairs; spores; faecal. anaerobin.	Diastase.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Light-yellow colonies; serrated edges.	Old distilled water.	Bolton.
.	Sulphur water.	Agardh.
Colonies, radiating from an oval centre like roots; later on colored yellow; slowly liquefy.	London Water-works.	Francland.
.	Stagnant water.	Warming.
Rapidly liquefy; little orange-yellow colonies, not growing in high temperature.	Air and water.	Koch.
Slowly growing; nail cultures; shining and orange-yellow; not liquefy.	Water.	Francland.
Round orange-yellow colonies, mostly on surface; slow growth; not liquefying.	Water.	Cohn.
Liquefy; bright golden layer on potato.	Exudate of pneumonia.	Mace.
Slow-growing, chrome-yellow, whetstone in shape; not liquefy.	Water and skin of eczema.	Adametz and Unna.
Do not liquefy; require glucose for growth.	Baltic Sea.	Fischer.
Creamy layer on surface of gelatin.	Putrid broth.	Cohn.
.	Maize.	Schröter.
Liquefy rapidly; gray veil on surface of potato.	Casein ppt. and changed into butyric acid; ammonia set free.	Air.	Hueppe.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
BUTYRICUM (amylobacter).	Clostridium.	Thick motile rods enlarging for the spores; obligat. aerobic.	Amyloid substance.
CÆRULEUS.	Bacillus.	Rods in long chains.	Blue pigment, not soluble in water, alcohol, or acid.
CANDICANS (candidus).	Micrococcus.	Masses of cocci.
CAROTARUM.	Bacillus.	Threads of rods that bend in various directions; oval spores.
CATENULA.	Bacillus.	Motile rods with spores.
CAUCASICUS.	Bacillus.	Motile rods, with spores in each end.
CERASINUS SICCUS.	Micrococcus.	Very small cocci, singly and in pairs; aerobic.	Cherry-red pigment.
CEREUS ALBUS.	Micrococcus.	Cocci in short chains and bunches, colored by Gram.
CEREUS FLAVUS.	Micrococcus.	Staphylo. and strepto., and in zooglæa, colored by Gram.
CHLORINUS.	Bacillus.	Large rods, motile, green-colored, due to chlorophyll; aerobic.	Green pigment, soluble in alcohol.
CHLORINUS.	Micrococcus.	Cocci in zooglæa.	Green pigment, soluble in alcohol and water.
CINNABAREUS.	Micrococcus.	Large oval cocci in pairs; aerobic.	Brown-red pigment; foul odor.
CITREUS.	Bacillus (asco.).	Straight and bent rods in bundles; motile.	Citron yellow pigment.
CITREUS.	Micrococcus.	Large round cocci in chains of eight and more.	Cream-colored pigment.
CITREUS CONGLOMERATUS.	Micrococcus.	Diplococci and tetrads; aerobic.
CLAVIFORMIS.	Bacillus (Tyrothrix).	Small rods; spores; true anaerobin.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Not cultivated.	Forms butyric acid in presence of lactic acid.	Air, earth, and water.	Prazmowski and Van Tiegham.
Liquefy; a deep-blue layer on potato.	Water.	Smith.
Not liquefy; nail-shaped in test-tube.	Air around old cultures.	Flügge.
Rapidly liquefy on surface, a network centre on potato; round, light gray; grow rapidly.	Cooked carrots and beets.	A. Koch.
.....	Causes albumin to ferment.	Old cheese.	Duclaux.
.....	Ferments milk, producing the kerry drink.	Kefyr; grain.	Kern.
On potato; rapidly-forming cherry-red scum, not developed on gelatin.	Water	List.
Not liquefy; small wax-like drops; thick gray layer on potato; growth rapid.	Pus.	Passet.
Not liquefy; dark-yellow colonies; wax-like appearance.	Pus.	Passet.
Liquefy; greenish-yellow colonies.	Water.	Engelman.
Yellow-green layer on gelatin.	Boiled eggs.	Cohn.
Not liquefy; slow growth; bright-red points.	Air and water.	Flügge.
Slow growth; after two weeks small yellow points which take various shapes on potato; citron-yellow layer; growth more rapid.	Skin in eczema.	Unna and Tommasoli.
Dirty cream-colored colonies, which are raised and moist.	Water.	List.
Lemon-yellow colonies.	Dust and blennorrhagic pus.	Bumm.
.....	Ferments milk, giving rise to alcohol.	Fermenting albumin.	Duclaux.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
CONCENTRICUM.	Spirillum.	Thick motile spirals with flagella; aerobic.
CORONATUS.	Micrococcus.	Cocci singly and streptococci; aerobic.
CORYZÆ.	Micrococcus.	Large diplococci with rounded ends, the contact surfaces flat.
CREPESCULUM.	Micrococcus.	Round and oval cocci, singly and in zooglœa.
CYANEUS.	Micrococcus.	Oval cells.	Blue pigment.
CYANOGENUS (blue milk).	Bacillus.	Motile rods in chains; spores; aerobic.	Alkali and a pigment deepened by acids.
DICHOTOMA.	Cladothrix.	Various forms—rods, spirals, and cocci, in long threads.
DIFFLUENS.	Micrococcus.	Oval cocci; aerobic.	Fluorescent pigment, soluble in water.
DISTORTUS.	Bacillus (Tyrothrix).	Motile rods; spores; aerobic.	Alkali.
DYSODES.	Bacillus.	Long and short rods; spores.	An odor resembling peppermint and turpentine.
ENDOPARAGOGICUM.	Spirillum.	Dry motile spirals, joined in peculiar shapes.
ERYTHROSPORUS.	Bacillus.	Motile rods and threads; spores, slender.	Greenish - yellow pigment.
FIGURANS (mycoides).	Bacillus.	Large motile rods; spores; long threads; aerobic.
FILIFORMIS.	Bacillus (Tyrothrix).	Short motile rods; spores in one end.
FISCHERI.	Bacillus.	Phosphorescence.
FITZLIANUS.	Bacillus.	Short rods in threads; spores as large as the rods.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Not liquefying; concentrically-disposed colonies; very slow growth; not growing on potato.	Putrefying blood.	Kitasato.
A halo formed around the colonies.	Air.	Flügge.
White, raised glassy colonies, at first like pneumococci, later culture flattened; not liquefying.	No pathogenic action.	Acute coryzal secretion.	Hajek.
Bluish-green colonies.	Putrefying infusions.	Cohn.
Not liquefying; small white colonies.	Changes milk to deep-blue color.	Cooked potatoes.	Cohn.
Cultivated in infusion of plants.	Air of certain countries.	Fuchs.
Do not liquefy; small granular, yellow, colonies; green fluorescence.	Water.	Cohn.
.	Milk made viscid and casein precipitated.	Air.	Schröter.
.	Air.	Duclaux.
.	Bread and yeast.	Zopf.
Does not liquefy; green fluorescence; white colonies.	Trunk of worm-eaten tree.	Sorokin.
Liquefying; root-like processes extending in the gelatin; feather form in test-tube.	Air and putrefying substances.	Cohn.
Not liquefying; requires peptone for growth.	Causes casein to be precipitated from milk.	Garden-earth.	Flügge.
Transparent on surface; dark centre in the deep; not liquefying.	Duclaux.
	Beyerinck.
	Produces ethylic alcohol in meat extract.	Unboiled hay-infusion.	Zopf.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
FLAVA.	Sarcina.	Small cocci in packets.	Pigment.
FLAVUS.	Bacillus.	Small rods; immotile.	Pigment.
FLAVUS DESIDENS.	Streptococcus.	Cocci and diplococci in chains; aerobic.	Yellow-brown pigment.
FLAVUS LIQUEFACIENS.	Micrococcus.	Cocci and diplococci in zooglœa.	Pigment.
FLAVUS TARDIGRADUS.	Micrococcus.	Cocci in short chains, and diplococci.	Chrome-yellow pigment.
FLUORESCENS FÆTIDUS.	Micrococcus.	Small diplococci.	Blue-green pigment; acids turn red.
FLUORESCENS LIQUEFACIENS.	Bacillus.	Short motile rods; very thin.	Green fluorescent pigment.
FLUORESCENS NIVALIS.	Bacillus.	Short rods; motile.	Blue-green pigment.
FLUORESCENS PUTRIDUS.	Bacillus.	Motile rods; short, with rounded ends.	Green fluorescent pigment.
FOERSTERI.	Cladotrix.	Threads twisted in spirals; very irregular.
FÆTIDUM.	Clostridium.	Rods of varying length; very motile; a large spore in one end; anaerobic.	Strong gas-production; very foul odor.
FÆTIDUS.	Micrococcus.	See <i>Crepesulum</i> , with	which it is identi
FUSCESCENS.	Sarcina.		
FULVUS.	Micrococcus.	Round cocci.
FUSCUS LIMBATUS.	Bacillus.	Short rods; very motile; facultatively anaerobic.	Brown pigment.
FUSIFORME.	Bacillus.	Spindle-shaped, with pointed ends.
GENICULATUS.	Bacillus (Tyrothrix).	Rods variable length; spores.	A bitter substance.
GIGANTEUS URETHRÆ.	Micrococcus.	Streptococci in thick knots.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Liquefying.	Vomited matter.	
Liquefying; yellow viscid colonies; foul odor.	Drinking-water.	Mace.
Yellow porcelain-white colonies.	Air and old cultures; water.	Flügge.
Liquefying rapidly; yellow colonies.	Air and old cultures; water.	Flügge.
Softens gelatin; yellow beads, isolated.	Air.	Flügge.
Little button-like colonies that later on sink in, surrounded by violet-green color; liquefying; growth rapid.	Post-nasal space.	Klamann.
Liquefying; white, sunken, iridescent colonies.	Water and air; conjunctival sac.	Flügge.
Quickly liquefying; growth rapid; small white points; later on, surrounded by blue-green fluorescence.	Colors the glacial waters green.	In snow and ice of Norway.	Schmolck.
Not liquefying; transparent at first, then green fluorescence and urinary odor.	All putrefactions.	Flügge.
.	Lachrymal canal.	Cohn.
Liquefying; growth rapid; small colonies that soon become filled up with fluid and assume a spherical form.	Old cheese and serum of mice inoculated with garden-earth.	Liborius.
cal.			
Conical rusty-red colonies.	Excrement of horse.	Cohn.
Small brown colonies, along needle-track little branches; not liquefy.	In foul eggs.	Scheibenzuber.
.	Spongy layer on sea-water.	Warning.
.	Air and milk.	Duclaux.
No growth on gelatin; on agar, thin drops; nearly transparent; very slow growth; in bouillon, a flaky precipitate.	Normal urine and urethra.	Lustgarten.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
GRAVEOLENS.	Bacillus.	Small rods, nearly as broad as they are long.	Foul gas.
HÆMATODES.	Micrococcus.	Cocci in little zooglœa.	Red pigment.
HANSENI.	Bacillus.	Medium large rods.	Yellow pigment; insoluble.
HYACINTH.	Bacillus.	Short rods in dumb-bell shapes.
HYALINA.	Sarcina.	Round cocci in groups of 4 to 24.
IANTHINUS.	Bacillus.	See <i>Bacillus violaceus</i> .	
INDICUS.	Bacillus.	Short motile rods; no spores; anærobin facul.	Scarlet pigment altered by heat.
INTESTINALIS.	Sarcina.	Very regular packets of cocci, eight in each.
JEQUIRITY.	Bacillus.	Medium-sized rods; spores.	Ferment called abrin.
KÜHNIANA.	Crenothrix.	Long threads, breaking up into cocci. They are ensheathed.
LACTEUS FAVIFORMIS.	Micrococcus.	Diplococci; not decolorized by Gram.
LACTIS ERYTHROGENES.	Bacillus.	Short immotile rods; round ends.	Yellow pigment and red pigment.
LEPTOMITIFORMIS.	Beggiatoa.	Filaments medium size.
LEUCOMELÆNUM.	Spirillum.	Two or three spirals; dark granular contents; clear spaces between.
LINEOLA.	Bacillus.	Short motile rods in zooglœa, with flagella.
LIODERMOS.	Bacillus.	Short motile rods; rounded ends.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Liquefying; irregular grayish, later greenish, colonies, with very foul odor.	Skin between toes.	Bordoni-Uffreduzzi.
Grows best on white of egg at 37° C.; red layer.	Sweat of man.	Zopf.
On potato, a yellow growth which changes with age.	Yellow skin of nutrient infusions.	Rasmussen.
.	Slime of diseased hyacinth-bulbs.	Wakker.
.	Marshes.	Kützing.
Liquefying; oval colonies; scarlet-colored.	Intestine of monkey.	Koch.
.	Intestine of fowls.	Zopf.
.	Ferment causes ophthalmia.	Infusion of jequirity bean.	Sattler.
Colonies brick-colored from oxide of iron.	Drinking-water of wells.	Rabenhorst.
Not liquefying; white colonies; grow well on potato.	Mucus of vagina and uterus.	Bumm.
Small, round yellow dots, later on cup-shaped, with rose-colored periphery; liquefying.	In red milk and faeces.	Hueppe and Grotenfeldt.
.	Sulphur waters.	Trévisan.
.	Water over rotting plants.	Perty.
Slimy layer on potatoes.	Stagnant water.	Müller.
Liquefying; transparent, then thick layer on potato; like gum.	Air and potatoes.	Flügge.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
LITORALIS.	Merismopedia.	Cocci in groups of fours, containing sulphur.
LITOREUS.	Bacillus.	Oval rods, never in chains or zooglœa.
LIVIDUS.	Bacillus.	Medium-sized rods; motile.	Deep blue-black pigment.
LUTEA.	Sarcina.	Cocci singly and in fours.	Pigment citron-yellow.
LUTEUS.	Bacillus.	Short immotile rods, with large oval spores.	Pigment; soluble in water; acids intensify.
LUTEUS.	Micrococcus.	Oval cocci.	Pigment, not acted upon by acid or alkali.
LUTEUS.	Micrococcus.	Diplococci very motile.	Yellow pigment, turning brown-red.
MAIDIS.	Bacillus.	Rods with pointed ends; very motile; seldom in threads; oval spores.
MARSH.	Spirillum.	See <i>Plicatile</i> .	
MEGATERIUM.	Bacillus.	Large motile rods; spores; ærobic.
MELANOSPORUS.	Bacillus.	Rods; ærobic.	Black pigment, not acted upon by acids or alkalies.
MERISMO-PEDI- OIDES.	Bacillus.	Threads of rods which are formed from cocci-like spores; zooglœa in packets.
MESENTERICUS FUS- CUS (potato).	Bacillus.	Small motile rods with spores.
MESENTERICUS VUL- GATUS (potato).	Bacillus.	Thick motile rods in threads; spores.	Diastase.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
.	Sea-water.	Oersted and Rabenhorst.
.	Sea-water.	Warming.
Ink-spot at first, slowly liquefying; blue-violet colored later on; slow growth.	Berlin Water-works.	Plogge and Proskauer.
Not liquefying; little elevations; citron-yellow centre; yellow layer on potato.	Air.	Schröter.
Not liquefying; irregular in form; golden-yellow colored.	Air.	Flügge.
Do not liquefying; small citron-yellow colonies on potato.	Air.	Schröter.
Round, light-yellow colonies, growing larger in a few days; on potato a slimy covering with mouldy odor; slowly liquefying.	Water.	Adametz.
Gray points in deep, veil-like on surface; liquefying; on potato, a wrinkled skin of brownish color.	In solutions of sugar an aldehyde produced.	In maize and in pellegra; fæces.	Paltauf and Heider.
Yellow irregular masses; thick layer on potato.	Cooked cabbage.	De Bary.
First gray, then black, pellicle.	Air and potatoes.	Eidam.
.	Stagnant water.	Zopf.
Liquefying; white colonies, ray-like periphery; brown layer on potato.	Potato.	Flügge.
Yellow colonies, dark centre, ciliary processes at periphery; brown layer on potato, penetrating the substance.	Coagulates milk and forms diastase out of starch.	Air and old potatoes.	Flügge.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
MESENTEROIDES.	Leuconostoc.	Masses of cartilaginous zoogloea, composed of rods and cocci; arthrospores.
MILLER'S.	Bacillus.	Delicate rods, slightly curved; immotile.
MINUTA.	Sarcina.	Cube-shaped packets.
MIRABILIS.	Beggiatoa.	Very wide threads, rounded ends and curled; sulphur granules.
MULTIPEDICULOSUS.	Bacillus.	Long, slender rods.
MULTISEPTATA.	Phragmidiothrix.	Long threads, containing cocci which are not free; they have no sulphur, and are not enclosed in a sheath.
NASALIS.	Micrococcus.	Diplococci, motile; also streptococci.
NAVICULA.	Bacillus.	Spindle-shaped rods.	Amyloid material
NITRIFICANS.	Micrococcus.	Small cocci.	Forms saltpetre.
NIVEA.	Beggiatoa.	Very thin filaments.
NODOSUS PARVUS.	Bacillus.	Rods formed at angles; immotile.
OBLONGUS.	Micrococcus.	Motile cocci, singly and in filaments; aerobic.
OCHROLEUCUS.	Micrococcus.	Cocci in pairs and packets; spores.	Yellow pigment.
PALUDOSA.	Sarcina.	Spherical, transparent, colorless cocci.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
.	Converts molasses into a gelatinous mass.	Beet-root juice.	Cienkowski.
Liquefies; not growing on the surface.	Caries of teeth.	Miller.
Grows slowly; reacts to iodine, turning blue.	Sour milk. Sea-water.	De Bary. Cohn.
Insect-shaped colonies.	Potatoes. Sea-water.	Flügge.
Grayish points, raised, opaque; rapid growth; not liquefying.	Nasal space and secretion. Potatoes. Soil.	Hack. Reinke and Berthold. VanTiegham
White flakes.	Sulphur waters.	Rabenhorst.
Slow growth at 37° C.; in agar a white line, which in the centre becomes porous.	Urethral secretion.	Lustgarten.
Grows best in cultures to which glucose and ammon. tartrate have been added.	Causes gluconic fermentation.	Beer.	Boutroux.
Liquefying; slow growth; thin yellow membrane; sulphurous odor.	Urine. Water from sugar-factory.	Prove. Schröter.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
PASTEURIANUS.	Bacillus.	Differs from bacil. aceti in that the cells contain an amyloid matter.
PFLÜGERI.	Bacillus.	Short rods in threads.	Phosphorescence.
PHOSPHORESCENS GELIDUS.	Bacillus.	Motile; round, short rods; aerobic.	Phosphorescence.
PHOSPHORESCENS INDICUS.	Bacillus.	Large motile rods.	Phosphorescence.
PHOSPHORESCENS, North Sea.	Bacillus.	Motile rods.	Phosphorescence.
PHOTOMETRICUS.	Bacillus.	Motile, red-colored rods.	Sulphur and red pigment caused by light.
PLICATILE.	Spirillum.	Long motile, thin spirals; round ends.
POLYMYXA.	Clostridium.	Motile rods in threads with spores.	Amyloid, colored blue by iodine.
PRODIGIOSUS.	Bacillus.	Short motile rods; aerobic.	Red pigment, soluble in alcohol trimethylamine.
PROTEUS MIRABILIS.	Bacillus.	Very motile, short rods; aerobic.
PROTEUS VULGARIS.	Bacillus.	Rods sometimes curved, as spirillum.
PROTEUS ZENKERI.	Bacillus.	Motile rods.
PSEUDO-DIPHTHERIÆ.	Bacillus.	Small rods, similar to the true bacillus; immotile.
PUTRIFICUS COLI.	Bacillus.	Slender motile rods; long threads; spores.
PYOGENES TENUIS.	Micrococcus.
RADIATUS.	Bacillus.	Motile rods with rounded ends; anærobic; oval spores.	Strong - smelling gas.
RADIATUS.	Streptococcus.	Small cocci in chains.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
.....	Heavy beers.	Hansen.
Not liquef'g; requires glucose; grows well on potato.	Putrid meat and fish.	Ludwig.
Not liquefying; grows best with glucose and salt.	Salt fish.	Förster.
Liquefying; grows best at 30° C.	Tropical seas.	Fischer.
Liquefying; colonies look as if punched out; grows best at 15° C.	Water around Kiel.	Fischer.
Movements depend upon light.	Engelman.
.....	Stagnant water.	Ehrenberg.
Thick skin on potato.	Causes fermentation in dextrin solutions.	Prazmowski.
Little red colonies; liquefying rapidly; especially abundant on potatoes.	Bread and potatoes.	Ehrenberg.
Liquefying slowly; opaque centre, irregular processes.	Putrefaction.	Hauser.
Liquefying quickly.	Putrefaction.	Hauser.
Not liquefying; thick white layer on potato.	Putrefaction.	Hauser.
Grows at ordinary temperature, rapidly forming on surface a brownish growth; pin-head colonies raised above surface; not liquefying.	Not virulent.	In diphtheritic membrane and normal pharynx.	Wellenhof.
.....	Decomposes albumen.	Human fæces.	Bienstock.
On agar, a glassy growth.	Closed abscesses.	Rosenbach.
Liquefying; growth rapid; colonies like moulds, from centre radiating in all directions and through the gelatin; the air must be excluded.	Not pathogenic.	In serum of white mice inoculated with earth.	Lüderitz.
Liquefying; white colonies with greenish tinge; funnel-shaped in test-tube.	Air.	Flügge.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
RAMOSUS LIQUEFACIENS.	Bacillus.	Motile rods.
REITENBACHII.	Merismopedia.	Cocci in packets or plates; colorless cell-wall containing chlorophyll.
ROSACEUS.	Micrococcus.	Large cocci in pairs and tetrads.	Red pigment.
ROSEA.	Sarcina.	Spherical cocci in cubical packets.
ROSEA PERSEINA.	Beggiatoa.	Long rods with coccishaped bodies in them, containing sulphur and a red pigment.	Pigment called bacterio-purpurin.
ROSEUM.	Spirillum.	Very short curved rods; motile and spores.	Pigment soluble in alcohol.
RUBER.	Bacillus.	Motile rods in groups.	Brick-red pigment.
RUBRUM.	Spirillum.	Motile; short spirilla; aerobic.	Pale-rose pigment.
RUFUM.	Spirillum.	Long motile spirals.	Red-rose pigment.
RUGULA.	Spirillum (vibrio).	Motile rods, in long spirals, singly and in chains, with flagella and spores; anærobic.
SAPROGENES.	Bacillus.	Large rods, terminal spores; facultatively anærobic.
SCABER.	Bacillus (Tyrothrix).	Short motile rods in chains; spores; aerobic.	Tyrosin and leucin are formed.
SCHEURLÉN'S.	Bacillus.	Short motile rods; spores.
SEPTICUS.	Bacillus.	Non-motile rods in threads and spores; anærobic.
SERPENS.	Spirillum.	Long, lively threads, with three windings.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Liquefying; concentric colonies; funnel-shaped in test-tube.	Air.	Flügge.
.	Caspary.
Not liquefying; small red knobs, with faecal odor.	Air.	Flügge.
.	Marshes.	Schröter.
.	Marshes.	Zopf.
Not liquefying; thick violet colonies; deep red on potato.	Blennorrhagic pus.	Mace.
.	Boiled rice.	Frank.
Not liquefying; grows slowly; pale-rose colonies.	Dead mice.	Esmarch.
.	Stagnant water.	Perty.
Liquefying rapidly; round yellow dots with zone; faecal odor.	Causes cellulose to ferment.	Vegetable infusions and tartar of teeth.	Müller.
Grows slowly; foul odor.	Putrefaction.	Rosenbach.
.	Duclaux.
Growth best at 39° C.; slowly liquefying on potato; a yellow, wrinkled skin, underneath which a red color.	In carcinomatous and normal mamma.	Scheurlen.
.	Putrid blood.	Klein.
.	Stagnant water.	Müller.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
SIMILIS.	Bacillus.	Immotile rods; transparent spores.
SPINOSUS.	Bacillus.	Large motile rods; spores; true anærobin.
SUBFLAVUS.	Micrococcus.	Diplococci colored by Gram's fluid.
SUBTILIFORMIS.	Bacillus.	Immotile rods in threads; transparent spores.
SUBTILIS (hay bacillus).	Bacillus.	Large motile rods, three times longer than broad, in threads, with flagella and spores; ærobie.
SYNCYANEUS.	Bacillus.	Same as <i>Cyanogenus</i> .	
SYNXANTHUS (yellow milk).	Bacillus.	Short, thin motile rods.	Yellow pigment, soluble in water; similar to aniline colors.
TENUE.	Spirillum.	Large motile spirals with flagella.
TENUIS.	Bacillus (Tyrothrix).	Motile rods in long chains; spores.
TERMO.	Bacillus.	Short motile, cocci-like rods in zooglyca.
TREMULUS.	Bacillus.	Motile rods with flagella and large round spores.
TUMESCENS.	Bacillus.	Short rods with spores.
TURGIDUS.	Bacillus (Tyrothrix).	Short immotile rods in long chains; spores; ærobie.	Carbonate of ammonium.
ULNA.	Bacillus.	Very large rods in chains and singly; not very motile; large spores.
UNDULA.	Spirillum.	Long motile spirals, with flagella.
UREÆ.	Bacillus.	Short rods; spores; ærobie.	Ferment, propylamine.
URINÆ.	Sarcina.	Small cocci in families.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Grows rapidly.	Human fæces.	Bienstock.
Liquefying; spiny periphery; foul odor due to methylmercaptan.	Albuminous decomposition.	Garden-earth.	Lüderitz.
Liquefying; yellow dots.	Vaginal secretion and lochial discharges.	Bumm.
Grows best at 37° C.	Human fæces.	Bienstock.
Liquefying; gray centre, wreath-like border; thick layer on potato.	Soil and dust, hay, etc.	Ehrenberg.
In boiled milk a yellow pigment is formed.	Boiled milk and potatoes.	Ehrenberg.
.	Stagnant water.	Ehrenberg.
.	Precipitates casein; forms a pellicle on milk.	Fermenting cheese and milk.	Duclaux.
Liquefying; opaque centre, yellow layer next, and the periphery lobed; funnel-shaped in test-tube.	Connected with putrefaction of plants.	Dujardin.
.	Putrefying plants.	
On boiled carrots a wrinkled gelatinous disk.	Boiled carrots.	Zopf.
A pellicle formed on surface of milk; a heavy precipitate beneath.	Fermenting milk and cheese.	Duclaux.
On boiled egg little zoogloea.	Putrefying water and boiled eggs.	Cohn.
.	Vegetable infusions.	Müller.
Resembling a globule of fat; grows well in mucous urine.	Splits urea into ammonii carbonas.	Stale urine.	Miquel.
.	Bladder.	Welcker.

NON-PATHOGENIC

Name.	Genus.	Biology.	Product.
UROCEPHALUS.	Bacillus (Tyrothrix).	Cylindrical motile rods with spores; anaëro- bic.
VENTRICULA.	Sarcina.	Cubical packets of 8 to 64 cocci.
VENTRICULI.	Bacillus.	Rods motile, often in bundles of four.
VERSICOLOR.	Micrococcus.	Small cocci.
VIOLACEUS.	Bacillus.	Motile rods, round end; spores.	Violet pigment, sol- uble in alcohol.
VIOLACEUS.	Bacillus.	Immotile rods, forming large spores.	Violet pigment, like aniline.
VIRENS.	Bacillus.	Straight rods; spores; immotile; green tinged.	Supposed to con- tain chlorophyll.
VIRESCENS.	Bacillus.	Short motile rods with flagella very broad.	Deep-green pig- ment, turning yellow-brown.
VIRGULA.	Bacillus (Tyrothrix).	Slender immotile rods; spores aerobic.
VIRIDIS.	Bacillus.	Little immotile rods; oval spore, which is tinged green.
VISCOSUS.	Bacillus.	Motile rods, rounded ends, usually in pairs.	Green pigment.
VISCOSUS.	Micrococcus.	Streptococci of globular cells.	Gummy substance, called viscosa, and ferment.
VITICULOSUS.	Micrococcus.	Oval cocci in large groups.
VOLUTANS.	Spirillum.	Long spirals with flagella.
ZOPFIL.	Bacillus.	Long motile rods, break- ing up into spores like cocci.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
.....	Fermenting milk.	Duclaux.
Not liquefying.	Contents of stomach.	Goodsir.
Round colonies with dark centre; slow growth; not liquefying.	Peptonizes albumen.	Stomach of dogs fed on meat.	Raczynssky.
Not liquefying; iridescent yellow surface.	Air.	Flügge.
Not liquefying; centre deep violet; color remains on agar a long time.	Water.	Zopf.
Liquefying; transparent colonies, surrounded by violet zone.	Boiled potato and water.	Schröter.
.....	Stagnant water.	VanTiegham.
Deep round colonies, the vicinity colored green; grows on surface; slow growth; not liquefying.	Green sputum.	Frick.
.....	Milk.	Duclaux.
.....	Water.	VanTiegham.
Rapid growth, liquefying; small hair-like processes from colonies; later on, viscid and in threads, with green fluorescence.	Water and earth.	Francland.
.....	Mucoid fermentation in wine and beer.	Beer and wine.	Pasteur.
Not liquefying; a fine network in the colony; mucoid layer on potato.	Air.	Flügge.
.....	Marshes.	Ehrenberg.
Not liquefying; forms thick coils like braided hair.	Intestinal contents of fowls.	Kurth.

Name.	Genus.	Biology.	Product.
AEROGENES CAPSULATUS.	Bacillus.	Usually found in pairs, resembling diplococci; capsulated; obligate anaerobe.	Gas with characteristic odor.
ALVEL.	Bacillus.	Rods with large spores.
AMYLOVORUS.	Micrococcus.	Oval cells, never in chains.	Forms butyric acid.
ANTHRACIS SYMPTOMATICI.	Bacillus.	Large slender rods with swellings at spore; anaerobic.	Rancid odor.
ANTHRAX.	Bacillus.	Straight rods, slightly concave ends; immobile; aerobic; spores.	Toxalbumin.
ARTICULORUM (diphtheriticus).	Micrococcus.	Oval cocci in long chains, identical with pyogenes.
BOMBYCIS.	Micrococcus.	Oval cocci in chains and zooglæa; motile.
BOTULINUS.	Bacillus.	Large rounded ends; motile; flagellated; anaerobe.	Butyric acid; and a powerful toxin.
BUBONIC PLAGUE.	Bacillus.	Short thick rods with indistinct capsule.
BUCCALIS.	Leptothrix.	Long threads in thick bundles, containing masses of cocci and spirals.
CATTLE PLAGUE (Texas fever).	See <i>Hemorrhagic Septi</i>	<i>cæmia</i> and <i>Swine</i>
CAVICIDA.	Bacillus.	Little rods twice as long as broad.	Propionic acid through decomposition of sugar.
CHAUVÆI (symptomatic anthrax), (Rauschbrand).	Bacillus.	Large rods with a spore at one end, assuming the clostridium type; motile; never in threads; true anaerobin.	Toxalbumin.

PATHOGENIC BACTERIA.

Culture Characters.	Actions.	Habitat.	Discoverer.
Acid reaction in litmus milk; coagulates casein with cavity-formation due to gas.	Causes fermentation; can produce gas from proteid alone.	Intestinal contents; earth; water; raw foods.	Welch.
Liquefying; growths radiating from centre downward; on potato a dry yellow layer.	Produces a disease in bees called "foul brood." "Fire-blight" in pear trees.	Larvæ of bees. 	Cheshire and Cheyne. Burrill.
Liquefy gelatin; grow only in atmosphere of hydrogen.	Causes quarter evil in animals.	Blood and tissues.	Bollinger.
Liquefying; granular colonies with irregular border; on potato a dry, creamy layer; in test-tube a thorny, prickly track.	Causes splenic fever in animals; malignant pustule in man.	Found in tissues and excreta of diseased animals.	Rayer and Davaine.
Grows well on gelatin; pale-gray colonies; not liquefying; slow growth on potato.	Fatal in mice and rabbits. Causes "flacherie" in silkworms.	Mucous membrane of diphtheria. Intestines of silkworms.	Löffler and Cohn. Béchamp.
Gelatin colonies appear as small semi-transparent spheres.	Sausage and meat poisoning.	Intestine of pig.	Van Ermen-gem.
Does not liquefy gelatin; white, point-like colonies turning gray and then brown.	Causes bubonic plague. Causes dental caries.	Tissues, body fluids, and secretions of plague patients. Teeth slime.	Yersin and Kitasato. Robin.
<i>Plague.</i>			
Not liquefying; irregular scale-like colonies, making the gelatin viscid.	Kills guinea pigs.	Human fæces.	Brieger.
Liquefying; opaque centre with ragged periphery; in test-tube growth below, with gas formation.	Causes "black leg," or Rauschbrand, in cattle.	Animals affected with disease.	Arloing, Carnevin, and Thomas.

Name.	Genus.	Biology.	Product.
CHOLERA ASIATICÆ	Spirillum.	Motile spiral-shaped rods, often in chains; very short flagella on ends, and strictly aerobic; spores have not been found.	Ptomaine-like muscarine; and toxalbumin, soluble in water.
CHOLERÆ GALLINARUM (chicken cholera).	Bacillus.	Immotile, cocci-like rods; without spores; strictly aerobic.	Toxalbumin.
CHOLERA NOSTRAS (Finckler).	Spirillum.	Motile, comma-shaped rods; strictly aerobic.
COLI COMMUNIS.	Bacillus.	Short motile rods, slightly curved, without spores; facultatively anaerobic.
CRASSUS SPUTIGENUS.	Bacillus.	Short, thick rods with rounded ends.
DECALVANS.	Micrococcus.	Spherical cells in great numbers.
DENTALIS VIRIDANS.	Bacillus.	Slightly curved rods, round ends.	Gray pigment.
DIARRHŒA OF INFANTS.	Bacillus.	Motile, medium-sized rods; spores; aerobic.	Toxalbumin.
DIARRHŒA OF MEAT-POISONING.	Bacillus.	Rods in groups of two and singly; round ends; spores.
DIPHThERIE.	Bacillus.	Immotile, middle-sized rods, rounded ends; facultat. anaerobic.	Toxalbumin.
DIPHThERIA OF CALVES (Vitulorum).	Bacillus.	Long rods in threads.
DIPHThERIA IN PIGEONS (Columbarum).	Bacillus.	Short rods in groups.
DIPLO BACILLUS OF CONJUNCTIVITIS.	Bacillus.	Non-motile; usually occurs in pairs.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Liquefying slowly, small depressed scars giving a frosted appearance, or like ground glass; on potato, a thin brown layer; in test-tube, a funnel-shaped liquefaction, with a bubble of air in the top, the funnel taking six or seven days to form well.	Causes cholera Asiatica in man and a similar trouble in animals.	Fæces of cholera patients.	Koch.
Not liquefying; small isolated white disks; in test-tube, a granular track; very faint.	Causes chicken cholera in fowls; not acting on man.	Blood and fæces of diseased fowls.	Pasteur.
Liquefying rapidly; colonies yellow-brown thick masses; in test-tube, funnel formed in 24 hours, dissolving all gelatin in two days; profuse gray mass on potato.	Harmless in man; fatal to guinea pigs.	Fæces of cholera nostras and caries of teeth.	Finckler and Prior.
Not liquefying; dark centre, undulated periphery; green-colored layer on potato; milky layer on surface of test-tube.	Fatal to guinea pigs and rabbits; causes diarrhœa in man; ferments sugar.	Fæces of nursing infants; water; choleraic stools.	Escherich.
Not liquefying; oval grayish, slimy colonies; nail-shaped growth in test-tube.	Mice and rabbits die in 48 hours with gastroenteritis.	Sputum.	Kreibohm.
.....	Causes alopecia areata.	In roots of hair.	Thin.
Not liquefying; round, sharply-outlined colonies, with bluish-gray opalescence.	Septic processes and death in mice and pigs.	In caries of teeth.	Miller.
Not liquefying; green colonies with foul odor.	Causes green diarrhœa in animals when intravenously injected, and is the cause of green diarrhœa in infants.	Fæces of infants suffering from green diarrhœa.	Lesage.
.....	Causes death in animals, with symptoms of septicæmia.	Blood and juices of choleraic diarrhœa.	Klein.
Not liquefying; little yellowish colonies; a membranous layer on potato.	Gives rise to diphtheria in man and animals.	Diphtheritic exudate.	Löffler.
.....	When inoculated in mice causes death.	Diphtheritic membrane of calf.	Löffler.
Whitish patches.	Necrosis in pigeons and other animals.	Diphtheritic membrane in pigeons.	Löffler.
Addition of blood-serum to media necessary; liquefying.	Found in subacute conjunctivitis.	Conjunctival secretion.	Morax.

Name.	Genus.	Biology.	Product.
DUCK CHOLERA.	Bacillus.	Similar to chicken cholera bacillus; immotile.
DYSENTERIAE.	Bacillus.	Resembles typhoid bacillus.	First slightly acid, then alkaline.
DYSENTERY (epidemic).	Bacillus.	Short motile rods; very thin.
ENTERITIDIS.	Bacillus.	Resembles typhoid bacillus.
ERYSIPELAS OF SWINE (Rothlauf; rouget du porc).	Bacillus.	Small, slender motile rods; facultatively anærobic.	Two vaccines, which give immunity.
FÆTIDUS OZÆNÆ.	Bacillus.	Short rods, very motile; in pairs and chains.	Foul gas.
FROG PLAGUE.	Bacillus.	See <i>Swine Plague</i>
GANGRENE.	Micrococcus.	Oval cocci in zoogloea.
GIGANTEA.	Leptothrix.	Long rods, cocci and short rods in one; thread also spiral.
GINGIVÆ PYOGENES.	Bacillus.	Short thick rods with rounded ends.
GLANDERS (Rotz, Mallei).	Bacillus.	Slender, immotile rods, usually singly; spores; facultatively anærobic.
GONORRHOÆ (Gonococcus).	Micrococcus.	Diplococci kidney-shaped; motile; do not color with Gram.
GROUSE DISEASE.	Bacillus.	Small rods and oval cocci in chains; immotile.
HÆMATOCOCCUS BOVIS.	Diplococcus.	Cocci seldom in chains; surrounded by a pale zone.
HÆMOPHILIA NEONATORUM.	Micrococcus.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Small round yellow colonies like wax-drops; not liquefying.	Fatal for ducks, but not for chickens or pigeons; less active than chicken cholera; causes diarrhœa and exhaustion.	Blood of diseased ducks.	Cornil and Toupet.
Resembles typhoid bacillus in many respects.	Produces one variety of dysentery.	In dysenteric stools.	Shiga.
Not liquefying; concentrically-arranged colonies; dry yellow membrane on potato.	The cause of epidemic dysentery in man; enteritis in guinea-pigs.	In fæces and mesenteric glands.	Chantemesse and Widal.
Resembles typhoid except that it ferments dextrose.	Produces enteritis in man and animals.	Intestinal contents; its toxin in meat of diseased animals.	Gaertner.
Very delicate silver-gray clouds on the gelatin, like bone-cells; not liquefying; in test-tube a very faint clouding.	Causes erysipelas in swine and other animals; the German "Rothlauf," French "rouget du porc."	Blood and organs of diseased animals.	Löffler.
Small greenish colonies which soon become liquefied and indistinguishable; a foul odor produced.	Mice are killed by injection; rabbits affected with progressive gangrene.	Secretion of persons suffering from ozæna.	Hajek.
. Grayish colonies with foul odor. Gangrenous tissue.	Eberth.
.	Causes caries of teeth.	Diseased teeth of animals.	Miller.
Growth rapid; liquefying; round colonies, visible to naked eye in 24 hours.	Fatal to mice, with septic processes.	Suppurating pulp of tooth.	Miller.
Light yellow, like honey, colonies, turning red-brown in a few days.	Glanders is caused by the bacillus in man and animals.	In epithelium and ulcerated glands.	Löffler.
Grow on blood-serum.	Gonorrhœa in man.	Gonorrhœal pus; in pus-cells and epithelium.	Neisser.
Not liquefying; small scales which turn gray in a few days, the edges serrated.	Fatal for mice and guinea pigs.	In blood and organs of diseased grouse.	Klein.
Best at 38° C.; not liquefying; small white points; sparse growth on potato; transparent.	Fatal for rabbits and rats; hyperæmia of lungs and spleen; blood-exudate in peritoneal cavity.	Blood and organs of animals diseased with hæmoglobinuria.	Babes.
.	Supposed to be the cause of the disease.	Found in this disease.	Klebs.

Name.	Genus.	Biology.	Product.
HÆMORRHAGIC SEPTICÆMIA (Infectious Pleuropneumonia, Wild Plague, German Swine Plague, Cattle Plague, Steer Plague, Rabbit Septicæmia).	Bacillus.	Short rods, twice as long as broad; immotile.
HOG CHOLERA (Swedish swine plague).	Bacillus.	Very motile oval rods, similar to hæmorrhagic septicæmia.	Peptonizes milk without coagulation.
INFLUENZA.	Bacillus.	Very minute rods or in clumps.
INSECTORUM.	Micrococcus.	Oval cells in chains and zooglœa; streptococci.
INTRACELLULARIS MENINGITIDIS.	Diplococcus.	Resembles gonococcus in morphology and arrangement in interior of leucocytes.
KOCH-WEEKS.	Bacillus.	Resembles influenza bacillus.
LACTIS ÆROGENES.	Bacillus.	Short, thick immotile rods.
LEPRÆ.	Bacillus.	Slender, immotile rods with pointed ends.
LIQUEFACIENS CONJUNCTIVÆ.	Micrococcus.	Single cocci; never in threads.
LUPUS.	Bacillus.	Same as <i>Tuberculosis</i> .	
MALIGNANT ŒDEMA (Gangrenous Septicæmia, Vibrio Septique).	Bacillus.	Large, slender rods, rounded ends, often in threads; motile, with flagella and spores; strongly anaerobic.	Soluble vaccine.
MAMMITIS OF COWS.	Micrococcus.	Oval cocci in chains; streptococci; facultatively anaerobic.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
White isolated pinhead points, not growing on potato; best at 37° C.; not liquefying.	A disease having different names in different animals, characterized by œdema, hæmorrhage, and septicæmia.	Blood and serum of diseased animals.	Hueppe.
Very good growth on gelatin and potatoes; a yellow-brown color.	In experiment, animal's death in four to eight days; bacteria in little emboli in capillaries.	Not spread through tissue, but in capillaries of diseased swine.	Salmon and Selander.
Grow best on blood-agar; colonies very small, almost transparent.	Produces epidemic influenza.	Secretions of respiratory tract.	Pfeiffer, Kitasato, Canon.
Transparent colonies, forming thin layer on Löffler's blood-serum and glycerine agar.	A contagious disease in the chinch-bug.	Stomach of chinch-bug.	Burrill.
Rarely grows, except on serum agar.	Causes epidemic cerebro-spinal meningitis.	In cerebro-spinal fluid and nasal secretions.	Weichselbaum.
Small porcelain-like disks with depressed centre; funnel-shaped in test-tube with gas.	Most common cause of acute contagious conjunctivitis.	Conjunctival secretion.	Koch and Weeks.
On blood-serum round white plaques with irregular borders.	Fatal to guinea pigs and rabbits; coagulates milk; decomposes sugary solutions.	Fæces of nursing infants and of cholera.	Escherich.
Liquefying; growth rapid; colonies on surface, with little radiating branches from a dark centre; those in deep, berry-shaped.	Causes leprosy in man and animals.	Leprous tissue.	Hansen.
Liquefying; thick centre, radiating periphery; in high culture in test-tube, gas-bubbles arise, with foul odor.	On cornea of rabbits causes slight clouding.	Normal human conjunctiva.	Gombert.
Not liquefying; brown, round granular colonies; grows slowly; in test-tube, heavy deposit along the needle's track.	Animals quickly die with extensive gangrene and œdema.	Garden-earth.	Pasteur.
	Causes contagious mastitis in cows; coagulates milk.	Mammary gland.	Nocard and Mollereau.

Name.	Genus.	Biology.	Product.
MAMMITIS OF SHEEP.	Micrococcus.	Streptococci and in fours.
MELITENSIS.	Micrococcus.	5 μ in diameter; occurs singly or in chains of two or more; said to be flagellated.
METCHNIKOVII.	Spirillum (vibrio).	Motile spirals with flagella; aerobic.	An alkaline vaccine which will cause immunity.
NEAPOLITANUS.	Bacillus.	Small immotile rods, with rounded ends; no spores; facultatively anaerobic.	Produces acids in gelatin cultures.
NOMÆ.	Bacillus.	Small rods, with rounded ends, growing often in long threads.
OXYTOCUS PERNICIOSUS.	Bacillus.	Short rods with round ends.
PARATYPHOID.	Bacillus.	Resembles typhoid bacillus.	Indol sometimes produced.
PNEUMONIA (Pneumococcus of Fränkländer).	Bacillus.	Short, immotile rods, singly or in diplococci, surrounded with capsule; no spores; not colored with Gram; facultatively anaerobic.
PNEUMONIA (Pneumococcus of Fränkel; Micrococcus of Pasteur).	Bacillus.	Short, oval rods, often in chains; immotile; no spores; in the tissue surrounded with capsule, colored with Gram; facultatively anaerobic.
PNEUMONICIS AGILIS.	Bacillus.	Short, thick motile rods in pairs.
PROTEUS SEPTICUS.	Bacillus.	Slightly curved rods, swelled in portions, sometimes in long threads; motile.	Foul gas.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Liquefying; round centres with zone of liquefaction; cone-shaped in test-tube.	Causes contagious gangrenous mammitis in sheep.	Found in the milk of diseased sheep.	Nocard.
Small, round, slightly raised disks; do not liquefy.	Causes Malta fever.	Best obtained from spleen.	Bruce.
Grows quickly; colonies, some like cholera Asiatica, others like cholera nostras; liquefying.	Causes vibriion septicæmia in guinea pigs and pigeons.	Fæces of fowls.	Gamaleia.
Not liquefying; thin pearl-like scales in several layers; wrinkled and mucous layers on potato.	Causes death in some animals; not the cause of cholera.	Cholera epidemic of Naples, 1884.	Emmerich.
Granular spherical colonies in the deep, flat on the surface; not liquefying; growth rapid; best at 35° C.	No action on mice or rabbits.	In necrotic tissue of noma.	Schimmelbusch.
Small yellow granular colonies; nail-culture in test-tube.	Intravenous injection causes death in mice and rabbits; turns milk acid.	Sour milk.	Wyssokow itsch.
Ferments glucose, but not lactose or saccharose; does not coagulate milk.	Causes continued fevers.	Intestinal contents.	Widal, Gwyn, Schottmüller.
Does not liquefy; grows quickly; a button-like colony; in test-tube, as if a nail driven in the gelatin with head on surface.	An accompaniment of pneumonia, not a cause; animals not affected.	Pneumonic and other sputum, and lung tissue.	Friedländer.
Does not liquefy; grows slowly; small, well-defined masses; in test-tube, little separate globules, one above the other.	Causes pneumonia in man, septicæmia in animals; also serous inflammations in man, as pleurisy, peritonitis, etc.	Sputum of lung affections and serous inflammations.	A. Fränkel.
Liquefying; dark granular colonies; thick sediment in test-tube.	Pneumonia in rabbits.	From rabbits' pneumonia.	Schon.
Growth rapid; liquefying; colonies have foul odor, are small, thick branches, but soon all liquid.	Fatal for mice in one to three days.	From a child dying of intestinal gangrene.	Babes.

Name.	Genus.	Biology.	Product.
PSITTACI (pernici- cious).	Micrococcus.	Streptococci and zoog- leæ.
PYOCYANEUS.	Bacillus.	Thin motile rods; fac- ultatively anærobic.	Pyocyanin, a non- poisonous pig- ment.
PYOCYANEUS β .	Bacillus.	Forms a brown-yellow pigment; otherwise identical with above.
PYOGENES (Strepto- coccus erysipela- tis—Fehleisen).	Micrococcus.	Streptococci and zoog- leæ.
PYOGENES ALBUS.	Micrococcus.	Staphylococci and streptococci; facul- tatively anærobic.
PYOGENES AUREUS (micrococcus of osteomyelitis— Becker).	Micrococcus.	Staphylococci and zoog- leæ; facultatively anærobic.	Ptomaine, toxal- bumin, and pig- ment.
PYOGENES CITREUS.	Micrococcus.	Same as <i>Pyogenes au- reus</i>
PYOGENES FÆTIDUS.	Bacillus.	Short motile rods in pairs.
PYOGENES TENUIS.	Micrococcus.	Cocci without definite arrangement.
RELAPSING FEVER (Obermeier).	Spirillum.	Long, wavy spirals; motile.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
.....	Causes disease in gray parrots.	In blood of parrot's disease.	Wolff.
Liquefying; large, flat colonies with greenish fluorescence; on potato, yellow-green skin, deeply coloring the pulp.	Fatal for animals; colors the dressings green.	Pus.	Gessard.
.....	Ernst.
Not liquefying; round punctiform colonies; slow-growing.	Suppuration and septicæmia in animals.	Pus.	Rosenbach.
Liquefying; white opaque colonies.	Suppuration and abscess.	Pus.	Rosenbach.
Liquefying; small colonies with a yellow-orange pigment in centre; yeast-like smell; a moist layer on potato.	Causes abscesses and suppuration in man and animals.	Pus.	Rosenbach.
Colonies, citron-yellow color.	Suppuration.	Pus.	Passet.
Not liquefying; mucous layer on potato; very thick; in test-tube, a slight layer on surface, and small points along the track.	Fatal to animals.	Pus.	Passet.
On surface, transparent; thin growth; grows slowly.	Pus of abscesses.	Rosenbach.
Cannot be cultivated.	Causes fever in man and animals, and is the cause of relapsing fever.	Blood of man during an attack of the disease.	Obermeier.

PATHOGENIC

Name.	Genus.	Biology.	Product.
RHINOSCLEROMA.	Bacillus.	See <i>Pneumococcus</i> of	Friedländer, with
SALIVARIUS PYOGENES.	Micrococcus.	Very small round cocci and staphylococci.
SALIVARIUS SEPTICUS.	Bacillus.	Short, immotile rods, encapsulated in pairs, sometimes long chain; aerobic.
SALIVARIUS SEPTICUS.	Micrococcus.	Cocci singly and in zooglæa; aerobic.
SAPROGENES No. II.	Bacillus.	Short rods; facultatively anærobic.	Foul gas.
SAPROGENES No. III.	Bacillus.	Very short rods: facultatively anærobic.	Foul gas.
SAPROGENES FÆTIDUS.	Bacillus.	Immotile rods; spores.	Foul gas.
SENILE GANGRENE.	Bacillus.	Thin rods: immotile; singly and in pairs; ends somewhat thickened; aerobic; spores.
SEPTICÆMIA AFTER ANTHRAX.	Micrococcus.	Motile streptococci.
SEPTICÆMIA OF MICE.	Bacillus.	Smallest bacillus known; immotile.
SEPTICÆMIA OF RABBITS (Cuniculicida).	Bacillus.	See <i>Hæmorrhagic Septicæmia</i> .	
SEPTICUS ACUMINATUS.	Bacillus.	Thin, lancet-shaped rods; very slender.
SEPTICUS AGRIGENUS.	Bacillus.	Very short rods.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
which it is identical.	Frisch.
Slowly liquefying; small white opalescent colonies.	Local abscess in animals.	Saliva.	Biondi.
Not liquefying; gray circular colonies; transparent zone; in test-tube, separated.	Fatal to animals.	Saliva of healthy persons.	Biondi.
Not liquefying; round colonies; separated dots in test-tube.	Fatal to animals.	Saliva of puerperal women.	Biondi.
Grows quickly; on agar, hyaline drops which quickly coalesce, and form a mucoid layer with a foul odor, that of perspiring feet.	Produces septicæmia in rabbits.	Sweat of feet.	Rosenbach.
Forms a fluid gray band on agar; odor of putrefaction.	Suppuration in rabbit.	Putrid marrow of bone.	Rosenbach.
Not liquefying; thin, transparent layer; putrid odor.	Rabbits killed with large doses.	Mesenteric glands of swine with erysipelas and of healthy swine.	Schottelius.
Round yellow colonies; liquefying in 36 hours; best growth at 37° C.	Causes gangrene in mice, similar to senile gangrene of man.	In gangrenous tissue and blood of senile gangrene.	Tricomi.
In bouillon virulence destroyed.	Septicæmia in rabbits, but not in chickens or guinea pigs.	Blood of animal dead from anthrax.	Charrin.
Not liquefying; small flocculent masses in the deep; grows very slowly; in the test-tube producing a faint cloud.	Septicæmia in house-mice, but not field-mice.	Putrefying liquids.	Koch.
At 37° C. on blood-serum small transparent plates; later on, turning yellow.	Pathogenic for rabbits and guinea pigs; fever; and bacilli in blood and organs.	Navel stump of child dead of septicæmia.	Babes.
Not liquefying; brown centre, a ring, then yellow zone.	Septicæmia in mice and rabbits.	Earth of recently-ploughed fields.	Nicolaler.

Name.	Genus.	Biology.	Product.
SEPTICUS LIQUEFACIENS.	Micrococcus.	Streptococci and diplococci.
SEPTICUS ULCERIS.	Bacillus.	Oval rods; motile.	Gas; no odor.
SEPTICUS VESICÆ.	Bacillus.	Rods always single; very motile; oval spores.
SMEGMA.	Bacillus.	Slender curved rods, identical with what was known as syphilis bacillus of Lustgarten.
SOFT CHANCRE.	Bacillus.	Minute oval rods, chiefly in groups or chains.
SPUTIGENUM.	Spirillum.	Curved, comma-shaped rods; motile.
SUBFLAVUS.	Micrococcus.	Diplococci like gonococci; colored by Gram.
SWINE PLAGUE (American and French).	Bacillus.	Motile, oval rods, similar to that of hog cholera.	Causes casein precipitate in milk and acid formation.
SYCOSIFERUS FCETIDUS.	Bacillus.	Short, straight immobile rods, often in threads.	On potatoes a foul odor.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Liquefying; a thin granular streak, the surface sunken in; later, cone-like, the walls covered with leaf-shaped colonies.	Pathogenic for mice and rabbits, producing œdema, in the serum of which the cocci abound.	Blood and organs of child dying of septicæmia.	Babes.
Liquefying; yellow colonies, taken up with gas later on.	An ulcer in inoculated animals, followed by paralysis and death.	In blood of child with gangrenous ulcer.	Babes.
Not liquefying; small pin-head colonies, growing slowly; never larger; a brown centre, yellow periphery.	Pathogenic for mice and rabbits, producing death.	In urine of cystitis.	Clado.
Not cultivated.	Normal preputial secretions.	Alvarez and Tavel.
Has not been cultivated.	Produces soft chancre.	In the sore.	Ducrey.
Not cultivated.	Causes death in animals.	In caries of teeth and saliva.	Lewis.
Growth slow; liquefying; on tenth day yellow points with thready boundary; on potato, a brown, thread-like growth after two weeks.	No result on mucous membrane; injected under skin, abscess results.	Normal secretion of vagina and urethra.	Bumm.
Not liquefying; growth similar to typhoid germ; on potatoes good growth.	Found in American and French swine plague, in frog plague, and Texas fever; animals affected locally.	Found in capillaries in little emboli; not spread in organs of diseased animals.	Billings, Rietsch, and Eberth.
Slow growth; not liquefying; after four days, little white points, which do not change for several weeks, then the superficial ones are mucous-like; nail growth; on potatoes, rapid growth.	On human skin causes eruption, vesicular around hairs, then it becomes pustular; similar to sycosis.	From sycosis of the beard.	Tommasoli.

PATHOGENIC

Name.	Genus.	Biology.	Product.
SYPHILIS (Spirocheta Pallida).	Spirocheta.	Small delicate spirals, difficult to stain.
TETANUS.	Bacillus.	Large, slender motile rods, with spores in one end, drumstick shape, often in threads; true anærobic.	Ptomaines, tetanine, tetanotoxine, spasmotoxine; also a toxalbumin.
TETRAGENUS.	Micrococcus.	Large round cells, united in groups, usually of four, and surrounded by a capsule; immotile; aerobic.
TIMOTHY GRASS.	Bacillus.	Extremely acid-fast; resembles tubercle bacillus; in cultures may show club formation and branches.
TOXICATUS.	Micrococcus.	Cocci singly and in pairs.
TUBERCULOSIS.	Bacillus.	Slender rods, usually in pairs; not motile; spores not definitely determined; facultatively anærobic.	Kochine or paratoline, a glycerin extract of the pure culture (tuberculin).
TYPHOID.	Bacillus.	Slender motile rods, sometimes in threads; flagella, but no spores; facultatively anærobic.	Typhotoxin and toxalbumin.
TYPHOID OF SWINE (swine plague).	See <i>Swine Plague</i> .	
TYROGENUM (Deneke's).	Spirillum (vibrio).	Spiral-shaped rods; aerobic.
XEROSIS.	Bacillus.	Similar to diphtheria bacillus.

BACTERIA.—CONTINUED.

Culture Characters.	Actions.	Habitat.	Discoverer.
Not cultivated.	Supposed to cause syphilis.	In tissue and secretions of syphilitics.	Schaudinn.
Liquefy gelatin slowly; colonies have radiated appearance; a thorny growth along the track in test-tube.	Produces tetanus in man and animals.	Earth and manure.	Nicolaier and Kitasato.
Not liquefying; little porcelain-like disks; thick slimy layer on potato.	Fatal to guinea pigs and white mice.	Found in cavernous phthisical lungs.	Gaffky.
Colonies visible in thirty-six hours, scale-like and grayish white.	May produce tubercles.	Infusions of timothy grass.	Moeller.
.	Supposed to be the cause of <i>Rhus</i> (poison ivy) poisoning.	Found in the <i>Rhus toxicodendron</i> .	Burrill.
Grows best on blood-serum and glycerin agar at 37° C., forming little white crumbs on the surface; under microscope a hairy matted coil is seen; growths on potatoes when air-tight have been obtained.	Causes tuberculosis, local and general, in man and lower animals.	In all organs and secretions of tubercular persons.	Koch.
Not liquefying: little whetstone-shaped yellow colonies in the deep, and leaf-shaped ones on the surface; on potato, a very transparent, moist layer.	Gives rise to enteric or typhoid fever in man.	Found in dejecta and spleen and urine of typhoid patients.	Eberth.
Liquefy rapidly; small round colonies; dark funnel-shaped liquefaction in test-tube.	Several animals have died from inoculations.	From old cheese.	Deneke.
Differs from diphtheria bacillus in not producing acid in bouillon.	Found in pathological conditions of conjunctiva, sometimes in normal eye.	Kuschbert and Neisser.

INDEX

- ABBÉ'S condenser, 26
Achorion Schönleinii, 210
Acid dyes, 30
Aërobins, facultative, 23
 obligate, 23
Aërobioscope, 219
Aëroscope, 216
African tick fever, 194
Agar-agar, 55
 blood-, 60
 bouillon, preparation, 55
 gelatin-, 57
 glycerin-, 57
 inoculation of, 62
 Japanese method of preparing, 56
 litmus, 60
 nutrient, preparation of, 55
Age, influence of, on bacteria, 25
Agglutination phenomenon, 131
 reaction for tubercle bacillus, 119
Agglutinins, 81
Agglutinogen, 82
Aggressins, 77
Air, bacteria in, 216, 219
 examination of, 216
Alcoholic solution, saturated, 32
Alkaline anilin-water solutions, 34
 methylene-blue, 32
 stains, 31
Amœba dysenteriaë, 195
Anaërobins, facultative, 24
 obligate, 23
Anilin dyes, 30
Anilin-oil water, 31, 32
Anilin-water dyes, 32
Animals as culture-media, 67
 experiments upon, 84
Anthrax, 105
 symptomatic, 196
Antisepsis, 233
Antiseptics, 233
Antitoxin of diphtheria, 128
 of pneumonia, 153
 of tetanus, 173
Antituberculous serum, 119
Arnold's steam sterilizer, 47
Artesian well water, 221
Arthrospores, 20
Arthrosporous bacteria, 22
Asbestos, 221
Asexual cycle in man, 185
Aspergillus flavus, 211
 fumigatus, 211
 glaucus, 211
Asporogenic bacteria, 22
Autoclave, 47
Autovaccines, 170

BACILLEN emulsion, 119
Bacillus, 17
 acidi lactici, 95
 aërogenes capsulatus, 180
 alvei, 204
 amylobacter, 97
 anthracis, 105
 avicius, 198
 Boas-Oppler, 96
 botulinus, 139
 butyricus, 96
 capsule, 156
 cœruleus, 99
 coli communis, 137
 diagnostic points of, 225
 in water, examination for,
 224
 enteritidis sporogenes, 182
 erythrosporus, 99

- Bacillus, feces, 135
 fluorescens, 163
 liquefaciens, 100
 geniculatus, 96
 hay, 94
 indicus, 92
 Koch-Weeks', 165
 lactis cyanogenus, 97
 erythrogenes, 98
 lepra, 120
 mallei, 121
 megaterium, 93
 melitophtharus, 204
 mesentericus vulgatus, 92
 Milzbrand, 105
 murisepticus, 202
 mycoides, 93
 Neapolitanus, 135
 oedematis maligni, 174
 of anthrax, 105
 of bluish-green pus, 163
 of bubonic plague, 177
 of chicken cholera, 198
 of cholera, 140
 in water, 143
 products of, 142
 of diphtheria, 123
 products of, 127
 of dysentery, 179
 of erysipelas of swine, 201
 of fowl septicemia, 198
 of glanders, 121
 of influenza, 157
 of leprosy, 120
 of malignant pustule, 105
 of red milk, 98
 of rhinoscleroma, 153
 of soft chancre, 177
 of splenic fever, 105
 of symptomatic anthrax, 196
 of syphilis, 120
 of tetanus, 170
 of typhoid fever, 120
 bacteria resembling, 135
 in blood, 136
 in water, 135
 products of, 136
 paracolon, 136
 paratyphoid, 136
 phosphorescens gelidus, 101
 indicus, 100
- Bacillus phosphorescens indigenus,
 100
 prodigiosus, 91
 psittacosis, 137
 pyocyaneus, 163
 B, 165
 ramosus, 93
 root, 93
 smegma, 120
 spinosus, 95
 subtilis, 94
 tuberculosis, 109
 products of, 118
 violaceus, 99
 Wurzel, 93
- Bacteria, 17
 asporogenic, 22
 action in causing disease, 75
 aërobic, 23
 anaërobic, 23
 cultivation of, 71
 Botkin's method, 74
 Buchner's method, 74
 Esmarch's method, 72
 Fränkel's method, 73
 Hesse's method, 72
 Liborius's method, 71
 Roux's method, 73
 Wright's method, 74
 arthrosporous, 22
 causing disease, 25
 colonies of, growth and appearance, 68
 distribution of, 22
 effects of, general, 76
 local, 76
 examination of, methods, 26
 fluorescence of, 25
 fluorescent, 99
 gas-forming, 25
 growth of, 23
 in air, 216, 219
 in blood, 232
 in conjunctiva, 230
 in ear, 231
 in genito-urinary passages, 232
 in intestine, 231
 in milk, 95
 in mouth, 230
 in nasal cavity, 231
 in skin, 229

- Bacteria in soil, 227
 in stomach, 231
 in urethra, 168
 in urine, 102
 in vagina, 168
 in water, 220, 223
 infectious, 75, 76
 influence of age on, 25
 of electricity on, 24
 of light on, 24
 of oxygen on life and growth,
 23
 of Röntgen rays on, 24
 of temperature on life and
 growth, 23
 life of, 23
 locomotion, 19
 method of counting, in a culture,
 87
 non-pathogenic, 25, 91
 in water, 99
 table of, 238-261
 odors from, 25
 of hemorrhagic septicemia, 200
 of milk, 228
 of pneumonia, 148
 of suppuration, 158
 origin, 22
 oxidation by, 24
 parasitic, 75
 pathogenic, 25, 75, 105
 for animals, 196
 table of, 262-279
 phosphorescence by, 25
 phosphorescent, 100
 pigmentation by, 25
 pyogenic, 76
 reduction by, 24
 reproduction, 19
 saccharolytic, 139
 saprophytic, 75
 sewage, examination for, 224
 specific, 76
 staining of, 30
 suppurative, 76
 tables of, 238-279
 types, 17
 unstained, examination of, 27
 vibratory movements, 19
 vital action, 24
- Bacterial treatment of sewage, 226
- Bactericidie du charbon, 105
 Bacteriologic examination of organs
 and cavities, 229
 Bacteriolysis, 81
 Bacterium acidi lactici, 96
 aërogenosum, 163
 Balticum, 101
 Fischeri, 101
 Pflügeri, 101
 syncyanum, 97
 termo, 205
 ureæ, 102
 Zopfii, 94
 Ball's forceps, 37
 Basic dyes, 30
 Beggiatoa, 101
 alba, 102
 Biedert's method of collecting
 tubercle bacilli, 115
 Bile-salt media, MacConkey's, 61
 Blastomycetes, 207
 Blastomycosis, 208
 Blood, bacteria in, 232
 coagulum, 60
 typhoid bacillus in, 136
 Blood-agar, 60
 Blood-capsule, Wright's, 89
 Blood-serum, 57
 coagulation of, 58
 in liquid state, preservation, 59
 mixture, Löffler's, 60
 nutrient, preparation of, 57
 sterilization of, 58
 Boas-Oppler bacillus, 96
 Boiled eggs, 62
 Boiling water, 222
 Borer, Fränkel's, 227
 Botkin's method of cultivation of
 anaërobic bacteria, 74
 Bouillon, agar-agar, preparation
 of, 55
 guinea-pig, 62
 Löffler's, 49
 sterilization of, 50
 Bouillon-gelatin, Koch-Löffler, 53
 Bovine farcin du bœuf, 215
 Bread mash, 53
 Brood-ovens, 57
 Broth, glucose, 50
 glycerin, 50
 Brownian movements, 19

- Bubonic plague, 177
 Buchner's method of cultivating anaërobic bacteria, 74
 Buerger's method of staining capsule, 43
- CALMETTE'S ophthalmic tuberculin reaction, 119
 Capsule bacillus, 156
 stain, 43
 Buerger's method, 43
 of Hiss, 35
 of Welch, 35
 Carbol-fuchsin, 31
 Carbol-thionin stain, 34
 Cell-contents, 18
 Cell-wall, 18
 Chancre, soft, 177
 Charbon symptomatique, 197, 198
 Charcoal sponge, 221
 Chemotaxis, 80
 Chicken cholera, 198
 Cholera, chicken, 198
 immunity of Pfeiffer, 143
 red, 142
 Cladotranches, 212
 Cladotrix, 101
 actinomyces, 212
 dichotoma, 101
 Clostridium butyricum, 97
 Clouding of gelatin, 54
 Coagulation of blood-serum, 58
 Colitis contagiosa, 138
 Colonies, growth and appearances, 68
 macroscopic appearance, 68
 microscopic appearance, 68
 Comma bacillus of cholera, 140
 Compound solutions, 31
 Conjunctiva, bacteria in, 230
 Conjunctivitis, epidemic, 165
 diplobacillus of, 166
 Cotton plugs or corks, 48
 Cover-glass preparations, 36
 Crenothrix, 101
 Kühniana, 101
 Cultivation, 44
 artificial, 44
 of anaërobic bacteria, 71
 Cultures, egg, fresh, 61
- Cultures, glass slide, 62
 methods of, 44
 nail, 150
 plate, 64
 potato-, 51
 pure, by boiling, 67
 rolled, 67
 smear, 63
 stab, 63
 sterilization of, 44
 stroke, 63
 test-tube, 63
 thrust, 63
 Cutting sections, 38
- DAUGHTER-CELL, 207
 Decolorizing agents, 31
 Deny's B. F. tuberculin, 119
 Deodorant, 233
 Dextrose, 60
 Disinfectants, 44, 233
 Diphtheria, 123
 streptococcus in, 129
 toxins of, 128
 Diplobacillus of conjunctivitis, 160
 Diplococcus albicans amplus, 169
 tardissimus, 169
 intracellularis meningitidis, 153
 lanceolatus, 151
 pneumoniae, 149, 151
 Drying specimens, 36
 Dunham's peptone solution, 61
 rosalic acid solution, 60
 Dysentery, 179
- EAR, bacteria in, 231
 Edema, malignant, 174
 Egg, boiled, 62
 cultures, fresh, 61
 Ehrlich's side-chain theory of immunity, 80
 Electricity, influence of, on bacteria, 24
 Elsner's typhoid medium, 60
 Endocarditis, 160
 Endospores, 20
 Enteric fever, 129
 Erysipelas, 160
 of swine, 201

- Esmarch's cubes, 52
 method of cultivating anaërobic bacteria, 72
 tubes, 67
 Experiments on animals, 84
- FECES bacillus, 135
 Fermentation tube, 62
 Smith's, 62
 Ferments, 25
 coagulating, 25
 diastatic, 25
 fat-splitting, 25
 hydrolytic, 25
 inverting, 25
 proteolytic, 25
 Filter, hot-water, 54
 Filtered water, 221
 Filtration, 236
 Finkler-Prior vibrio, 146
 Fishing, 69
 Flagella, 19
 stain, with Löffler's mordant, 43
 Fluid media, 49
 Fluorescence, 25
 Fluorescent bacteria, 99
 Foods as source of infection, 228
 Forceps, Ball's, 37
 Formaldehyd, 235
 Fowl septicemia, 198
 Fränkel's borer, 227
 method of cultivating anaërobic bacteria, 73
 of staining tubercle bacilli, 113
 Fungi, staining, Unna's method, 43
 thrush, 208
- GABBET'S acid blue stain, 33
 Gametes, 186
 Gas-formation, 25
 Gelatin, 53
 clouding, 54
 inoculation of, 62
 modification of, 55
 potato-, 60
 sterilizing, 55
 Gelatin-agar, 57
 Gelatinous membrane, 18
 Genito-urinary passages, bacteria in, 232
- Germicides, 234
 Germination, 22
 Giemsa stain, 65
 Glanders, 121
 Glass plates, 64
 slide cultures, 62
 Glossina morsitans, 192
 palpalis, 192
 Glucose broth, 50
 Glycerin agar, 57
 broth, 50
 Gonococcus, 166
 urine media for, 61
 Gonorrhœa, 166
 Gram's iodine solution, 33
 method of double staining, 40
 of tissue staining, 41
 Gruber-Widal blood-serum test, 131
 Guinea-pig bouillon, 62
- HANDS, sterilization of, 236
 Hanging drop, 29
 Hay bacillus, 94
 Heat, 31, 234
 as disinfectant, 44
 dry, 45
 moist, 45
 Hemolytic serum, 81
 Hemorrhagic septicemia, 200
 Hesse's method of collecting bacteria from air, 216
 of cultivating anaërobic bacteria, 72
 Hiss' capsule stain, 35
 typhoid medium, 61
 Hoffman's pseudobacillus, 126
 Hot-air oven, 45
 Hot-water filter, 54
 Hueppe's fresh egg cultures, 61
- IMMUNE body, 81
 Immunity, 78
 acquired, 78
 active, 78
 Ehrlich's side-chain theory of, 80
 from injections of sterilized products of bacteria, 79
 from inoculations of small doses of very virulent bacteria, 79

- Immunity from inoculations with
 attenuated or weakened cultures of bacteria, 78
 inherited, 79
 natural, 78
 passive, 79
 phagocytic or cellular theory of, 80
 theories of, 79
 summary, 83
 unit, 129
- Impression of colonies, 69
- Incubators, 57
- Infection, 75
 conditions necessary to produce, 75
- Infectious bacteria, 75, 76
 diseases, 25
- Influenza, 157
- Infusoria, 184
- Inoculation, manner of, 52
 of agar, 62
 of animals, 84
 of gelatin, 62
- Insecticides, 235
- Instruments, sterilization of, 28
- Intestine, bacteria in, 231
- Iodin, 31
- Iris blender, 27
- JAPANESE method of preparing agar, 56
- Jenner's stain, 36, 189
- KLATSCH preparations, 69
- Klein's method of staining spores, 42
- Koch's alkaline methylene-blue, 32
 rules in regard to bacterial cause of disease, 87
 steam-chest, 46
- Koch-Löffler bouillon-gelatin, 53
- Koch-Weeks bacillus, 165
- Kühne's stain, 33, 42
- LACTOSE, 60
- Leishman's stain, 35
- Lepra bacillus, 120
- Leprosy, 120
- Leptothrix buccalis, 101
- Leukocytes, washed, 89
- Liborius's method of cultivating anaërobic bacteria, 71
- Life-cycle of malarial sporozoa, 184
 of protozoa, 184
- Light, influence of, on bacteria, 24
- Litmus-agar, 60
- Löffler's alkaline methylene-blue, 32
 blood-serum mixture, 60
 bouillon, 49
 mordant for flagella, 33, 43
 stain for bacillus of glanders, 122
- Lysins, 81
- MACCONKEY'S bile-salt media, 61
- Macrocytases, 80
- Macrogamete, 187
- Macrophages, 80
- Madura foot, 214
- Malaria, 184
- Malarial organisms, method of examination for, 189
 protozoa, estivo-autumnal form, 187
 quartan form, 187
 tertian form, 187
 sporozoa, life-cycle of, 184
- Mal de pis, 203
- Malignant edema, 174
 pustule, 105
- Mallein, 123
- Malta fever, 181
- Marchoux's thionin stain, 189
- Mastigophora, 184
- Media, fluid, 49
 neutralization of, 49
 Schultz's method, 50
 nutrient, 49
 solid, 51
 transparent, 53
- Mediterranean fever, 181
- Merozoites, 185
- Metchnikoff's theory of immunity, 80
- Methylene-blue, alkaline, 32
- Microbe en huit, 198

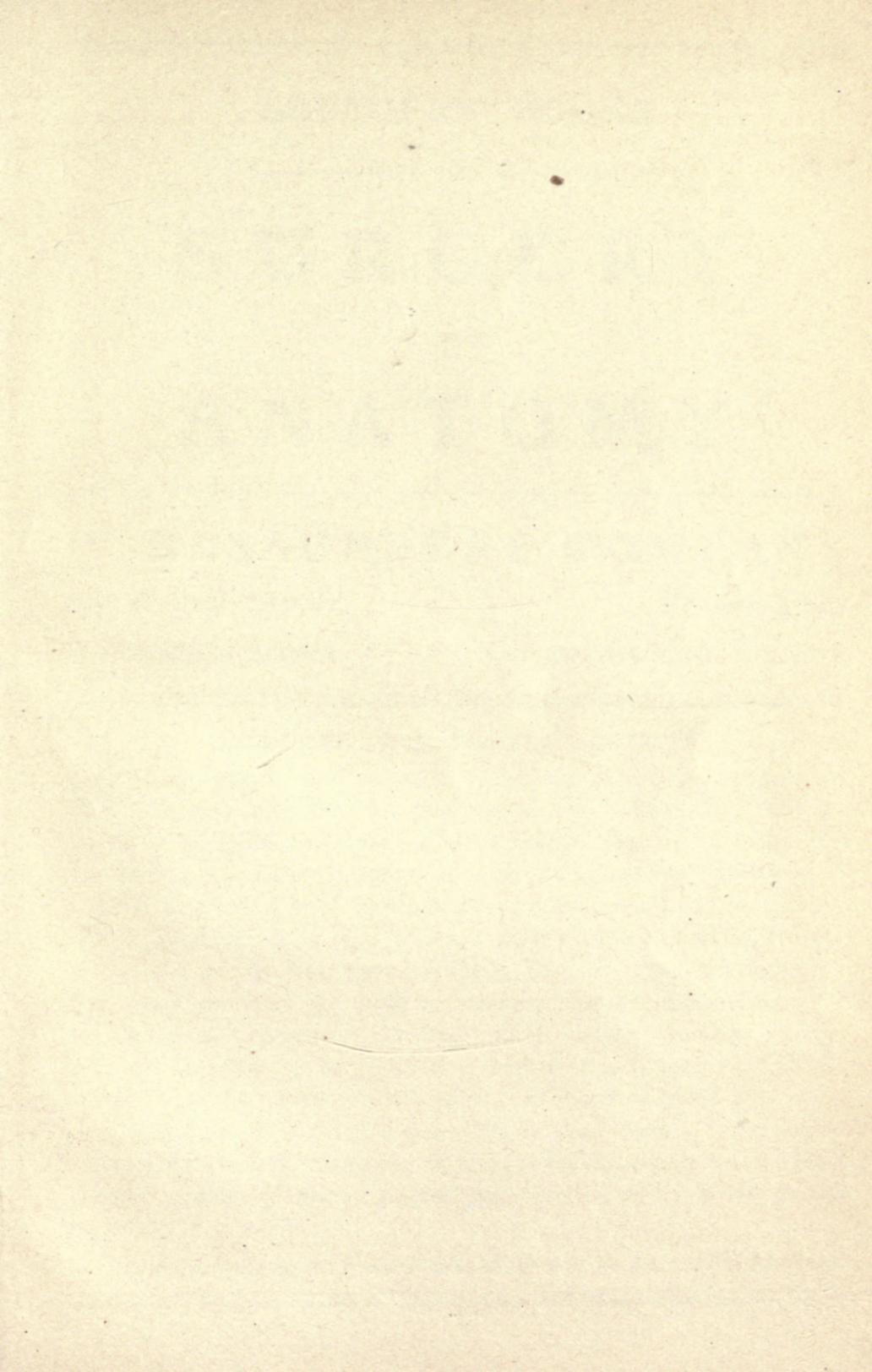
- Micrococcus, 17
 cereus albus, 162
 flavus, 162
 cholera gallinarum, 198
 citreus conglomerata, 168
 gonorrhœæ, 166
 indicus, 92
 melitensis, 181
 of mal de pis, 203
 of sputum septicemia, 151
 pasteuri, 151
 pyogenes citreus, 162
 tenius, 162
 subflavus, 169
 tetragenus, 155
 ureæ, 103
 Microcytases, 80
 Microgametes, 187
 Microgametocytes, 187
 Microphages, 80
 Microscope, 26
 Microsporion furfur, 211
 Microtome, 39
 Milk, bacteria in, 95, 228
 culture-medium, 61
 examination of, for tubercle
 bacilli, 114
 in stained specimen, 98
 Milzbrand bacillus, 105
 Moist chambers, 66
 Molds, 206
 examination of, 212
 true, 209
 Mordants, 31
 Mosquito, sexual cycle in, 187
 Mouse septicemia, 202
 Mouth, bacteria in, 230
 Mucor mucedo, 209
 Mycetoma, 214
- NAGANA, 191
 Nail cultures, 150
 Nasal cavity, bacteria in, 231
 Neapolitanus bacillus, 135
 Negri bodies, 193
 Neisser's stain for diphtheria, 34
 for gonococcus, 167
 Neutralization of media, 49
 Schultz's method, 50
 Nicolle's stain, 34
- Nitrification, 24
 Nitrifying organisms in soil, 227
 Nivellier leveling and cooling
 apparatus, 65
 Nutrient agar, preparation, 55
 blood-serum, preparation, 57
 media, 49
- ODORS from bacteria, 25
 Oidium, 207
 albicans, 208
 coccidioides, 208
 lactis, 207
 mycosis, 208
 Oil immersion, 26
 Ophthalmic tuberculin reaction of
 Calmette, 119
 Opsonic index, 83, 89
 technic, 87
 Opsonins, 82
 Oven, hot-air, 45
 Oxygen, influence of, on life and
 growth of bacteria, 23
- PARACOLON bacillus, 136
 Parasites, 23
 Parasitic bacteria, 75
 Paratyphoid bacillus, 136
 Park's method of cultivating
 anaërobic bacteria, 74
 Pasteur-Chamberland filter, 221
 Penicillium glaucum, 209
 Peptone solution, Dunham's, 60
 Petri saucers, 66
 method of collecting bacteria
 from air, 218
 Pfeiffer's cholera immunity, 143
 Phagocytosis, method of measuring,
 88
 Phagolysis, 80
 Phenol, 235
 solutions, 33
 Phosphorescence, 25
 Phosphorescent bacteria, 100
 Pigmentation, 25
 Pink-eye, 165
 Piroplasma bigeminum, 192
 bovis, 192
 Plague, bubonic, 177

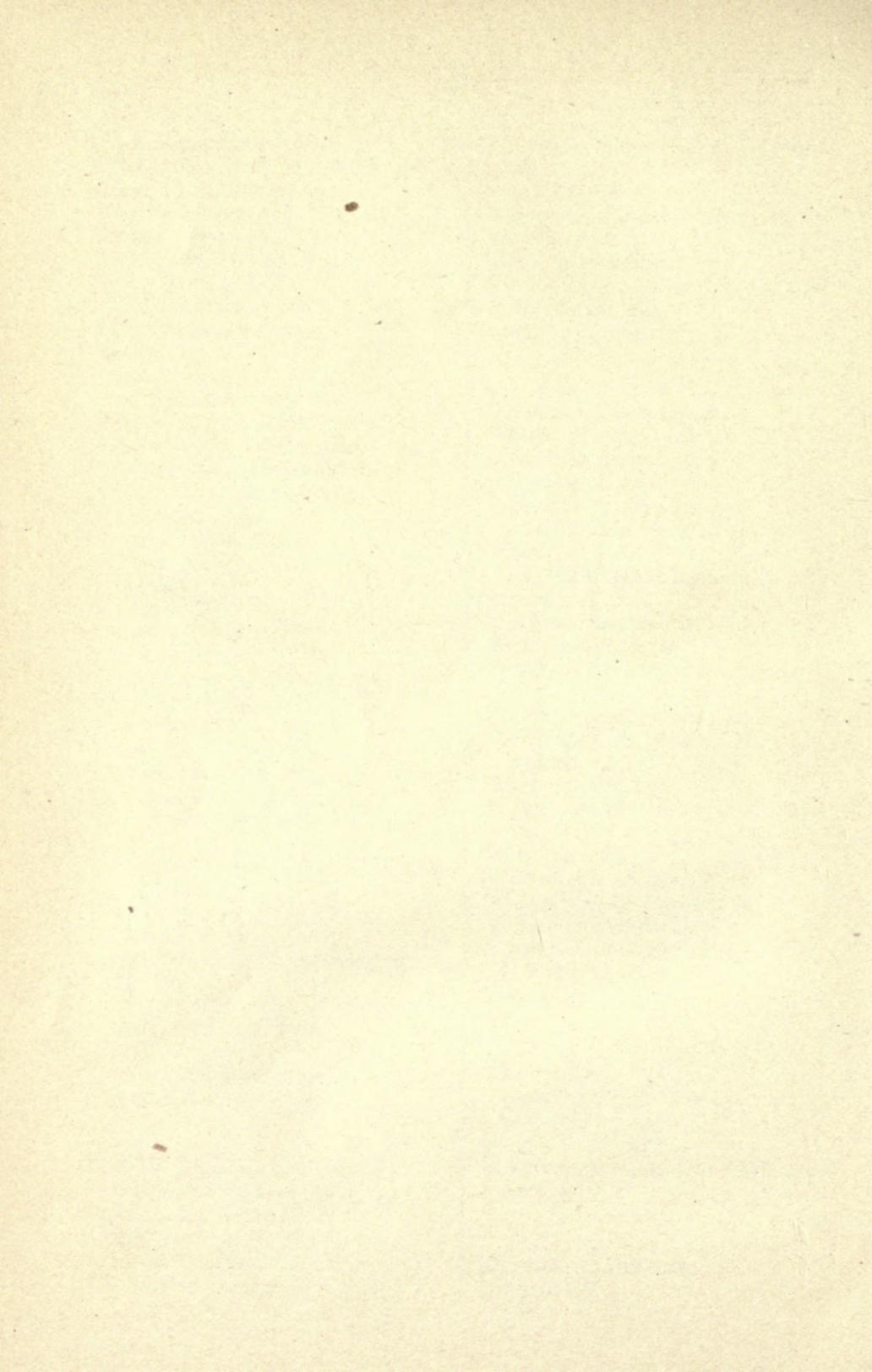
- Plate cultures, 64
 Pneumobacillus, 150, 151
 Pneumococcus, 150
 Pneumonia, 148
 Potassium iodid medium, 60
 Potato mash, 52
 Potato-cultures, 51
 Potato-gelatin, 60
 Potatoes, test-tube, 52
 Precipitins, 82
 Preservatives, 234
 Proteins, 25
 Proteus mirabilis, 205
 vulgaris, 205
 zenkeri, 205
 Protozoa, 183
 life-cycle of, 184
 malarial, estivo-autumnal form
 of, 187
 quartan form of, 187
 tertian form of, 187
 Pseudobacillus of Hoffman, 126
 Ptomäins, 24, 77
 Puerperal fever, 160
 Pustule, malignant, 105
 Putrefaction, 25
- RAUSCHBRAND, 198**
 Ray-fungus, 212
 Relapsing fever, 193
 Removing excess of stain, 37
 Rhinoscleroma, 153
 Rolled cultures, 67
 Romanowsky's stain, 35
 Röntgen rays, influence of, on
 bacteria, 24
 Root bacillus, 93
 Rotz-bacillus, 121
 Rouget du porc, 201
 Roux's double stain, 34
 method of cultivating anaërobic
 bacteria, 73
 test-tube, 52
- SACCHAROLYTIC bacteria, 139**
 Saccharomyces albicans, 207
 cerevisiæ, 206
 mycoderma, 207
 niger, 207
 rosaceus, 207
- Saccharomycetes, 206
 Salts of metals, 234, 235
 Sapremia, 76
 Saprophytes, 23
 Saprophytic bacteria, 75
 Sarcina, 104
 aurantica, 105
 lutea, 104
 ventriculi, 105
 Sarcodina, 184
 Schizogony, 184
 Schizomycetes, 17
 Schizophyceæ, 17
 Schizophyta, 17
 Schultz's method of neutralization
 of media, 50
 Schweinerotlaufbacillus, 201
 Sedgwick-Tucker method of col-
 lecting bacteria from air, 219
 Septicemia, 76
 fowl, 198
 hemorrhagic, 200
 mouse, 202
 Serum, antituberculous, 119
 hemolytic, 81
 Sewage bacteria, examination for,
 224
 bacterial treatment of, 226
 Sexual cycle in mosquito, 187
 Skin, bacteria on, 229
 examination of, 229
 Sleeping sickness, 192
 Small-pox, 196
 Smear culture, 63
 Smegma bacillus, 120
 Smith's fermentation tube, 62
 Soap, 236
 Soil, bacteria in, 227
 examination of, 227
 Solid media, 51
 transparent media, 53
 Soor, 208
 Spatula, 39
 Spirillum, 17, 103
 cholerae, 140
 bacteria similar to, 146
 concentricum, 103
 Finkleri, 146
 of relapsing fever, 193
 rubrum, 103
 tyrogenum, 147

- Spirochæta Obermeieri, 193
 pallida, 194
 Spironema pallidum, 194
 Splenic fever, 105
 Sporangium, 210
 Spores, arthro-, 20
 contents of, 20
 endo-, 20
 formation of, 20
 requisites, 21
 resistance of, 22
 staining of, 41
 Klein's method, 42
 Kühne's method, 42
 Weigert's method, 43
 Sporocyte, 185
 Sporogony, 184
 Sporozoa, 184
 malarial, life-cycle of, 184
 Stab culture, 63
 Stain, alkaline, 31
 capsule, 43
 Buerger's method, 43
 flagella, with Löffler's mordant,
 43
 Fränkel's, for tubercle bacillus,
 113
 Gabbet's acid blue, 33
 Giemsa's, 35
 Gram's iodine, 33
 Jenner's, 36, 189
 Koch's, 32
 Kühne's, 33
 Leishman's, 35
 Löffler's, 32
 for bacillus of glanders, 122
 mordant, 33
 Marchoux's, 189
 Neisser's, 34, 167
 Nicolle's, 34
 removing excess of, 37
 Romanowsky's, 35
 Roux's double, 34
 Unna's methyl-blue, 33
 Wright's, 190
 Ziehl-Neelsen, 33
 Staining, 30
 fungi, Unna's method, 43
 general method, 36
 Gram's double method, 40
 method for tissues, 41
 Staining of spores, 41
 Klein's method, 42
 Kühne's method, 42
 Weigert's method, 43
 of tissue sections, 37, 38
 solutions, 30
 special methods, 40
 tubercle bacilli in tissue, 116
 Staphylococcus, 18
 pyogenes albus, 162
 aureus, 160
 Steam sterilizer, Arnold's, 47
 superheated, 234
 Steam-chest, Koch's, 46
 Stegomyia fasciata, 196
 Sterilization, fractional, of Tyn-
 dall, 47
 of blood-serum, 58
 of bouillon, 50
 of cultures, 44
 of hands, 236
 of instruments, 28
 Sterilizer, Arnold's, 47
 Sterilizing gelatin, 55
 Stomach, bacteria in, 231
 Streptococcus, 18
 erysipelatis, 159
 in diphtheria, 129
 puerperalis, 160
 pyogenes, 159
 Streptothrices, 212
 Streptothrix actinomyces, 212
 farcinica, 215
 maduræ, 214
 Stroke culture, 63
 Sulphur dioxide, 235
 Suppuration, 158
 Suppurative bacteria, 76
 Susceptibility, 75, 76
 acquired, 76
 inherited, 76
 Syphilis, 194
 bacillus, 120

 TEMPERATURE, influence of on
 life and growth of bacteria, 23
 Test-tube, 48
 cultures, 63
 potatoes, 52
 Tetanus, 170

- Thermoregulators, 59
 Thermostat for blood-serum, 58
 Thread reaction, 82
 Thrush fungus, 208
 Thrust culture, 63
 Tick fever, African, 194
 Tissue preparations, 38
Torula cerevisiæ, 206
 Toxalbumins, 25, 77
 Toxins, 25
 nature of, 77
 of diphtheria, 128
 Toxone of diphtheria, 128
Treponema pallidum, 194
Trichophyton tonsurans, 210
Trypanosoma Brucei, 191
 Castellani, 192
 equiperdum, 192
 Evansi, 192
 hominis, 192
 Lewisi, 191
 neprevi, 192
 Rougetii, 192
 ugandense gambiense, 192
 Trypanosomes, 190
 Trypanosomiasis, 192
 Tsetse-fly disease, 191
 Tubercle bacillus, 109
 products of, 118
 Tuberculin, Deny's B. F., 119
 R, 118
 reaction, ophthalmic, of Calmette, 119
 Tuberculocidin, 118
 Tuberculosis, 109
 Tyndall, fractional sterilization of, 47
 Typhoid fever, 129
 medium, Elsner's, 60
 of Hiss, 61
 fever, Widal reaction in, 131
 Typhotoxin, 136
- UNNA'S borax methyl-blue, 33
 method of staining fungi, 43
 Urethra, bacteria in, 168
 Urine, bacteria in, 102
 media for gonococci, 61
Urobacillus liquefaciens, 103
- VACCINES, protective, in pus infections, 170
 Vaccinia, 196
 Vagina, bacteria in, 168
 Variola, 196
Vibrio Metchnikovi, 147
Vibrio butyrique of Pasteur, 97
 septicemia, 148
 septicæ, 174
- WASHED leukocytes, 89
 Water, artesian well, 221
 bacteria in, 220, 223
 boiling of, 222
 cholera bacillus in, 143
 examination of, 220, 223
 filtered, 221
 non-pathogenic bacteria in, 99
 purity of, 220
 typhoid bacillus in, 135
 Weak solutions, 32
 Weigert's method of staining spores, 43
 Welch's capsule stain, 35
 Wertheim's method of cultivating gonococcus, 166
 White mouth, 208
 Widal reaction in typhoid fever, 131
 Wire cage, 48
 Wolfhügel's apparatus, 225
 Wright's blood-capsule, 89
 chromatin stain, 190
 method of cultivating anaërobic bacteria, 74
 Wurzel bacillus, 93
- YEASTS, 206
 examination of, 212
 pathogenic, 207
 Yellow fever, 196
 Yokote's method of preparing agar, 56
- ZIEHL-NEELSEN stain, 33
 Zoöglea, 18





SAUNDERS' BOOKS

on

SURGERY

and

ANATOMY

W. B. SAUNDERS COMPANY

925 Walnut Street

Philadelphia

9, Henrietta Street

Covent Garden, London

SAUNDERS' REMARKABLE SUCCESS

WE are often asked to account for our extraordinary success. We can but point to modern business methods, carefully perfected business machinery, and unrivalled facilities for distribution of books. Every department is so organized that the greatest possible amount of work is produced with the least waste of energy. The representatives of the firm are men with life-long experience in the sale of medical books. Then, too, we must not overlook that major force in the modern business world—**advertising**. We have a special department devoted entirely to the planning, writing, and placing of advertising matter; and we might mention that the money annually spent in advertising now far exceeds the entire annual receipts of the House during its earlier years. These extraordinary facilities for disposing of large editions enable us to devote a large amount of money to the perfecting of every detail in the manufacture of books.

A Complete Catalogue of our Publications will be Sent upon Request

Keen's Surgery

AN UNABRIDGED TREATISE FOR THE SURGEON AND
THE GENERAL PRACTITIONER

IN FIVE OCTAVO VOLUMES

Surgery: ITS PRINCIPLES AND PRACTICE. Written by 66 eminent specialists. Edited by W. W. KEEN, M. D., LL. D., HON. F. R. C. S., ENG. AND EDIN., Emeritus Professor of the Principles of Surgery and of Clinical Surgery at the Jefferson Medical College, Philadelphia. Five large octavo volumes of over 1000 pages each, containing 2500 text-illustrations and 50 colored plates. Per volume: Cloth, \$7.00 net; Half Morocco, \$8.00 net.

VOLUME III JUST READY—VOLUMES IV AND V NOW IN PRESS
WITH 2500 TEXT-CUTS AND 50 COLORED PLATES

THE EMINENT CONTRIBUTORS

- | | | |
|-----------------------------|--------------------------------|-----------------------------------|
| Robert Abbé, M.D. | D. N. Eisendrath, M.D. | J. G. Mumford, M.D. |
| J. G. Adami, M.D. | Wm. L. Estes, M.D. | John C. Munro, M.D. |
| E. Wyllys Andrews, M.D. | J. M. T. Finney, M.D. | John B. Murphy, M.D. |
| G. E. Armstrong, M.D. | John A. Fordyce, M.D. | E. H. Nichols, M.D. |
| Thomas L. Bennett, M.D. | Chas. H. Frazier, M.D. | A. J. Ochsner, M.D. |
| A. D. Bevan, M.D. | Leonard Freeman, M.D. | William Osler, M.D. |
| Warren S. Bickham, M.D. | Frederick H. Gerrish, M.D. | Edmund Owen, F. R. C. S. |
| John F. Binnie, M.D. | John H. Gibbon, M.D. | Jos. Ransohoff, M.D., F. R. C. S. |
| George E. Brewer, M.D. | George Gottstein, M.D. | Brig. Gen. R. M. O'Reilly |
| T. A. Cabot, M.D. | Ludvig Hektoen, M.D. | Admiral P. M. Rixey |
| Hampton L. Carson, Esq. | Orville Horwitz, M.D. | John B. Roberts, M.D. |
| E. A. Codman, M.D. | Albert Kocher, M.D. | Mayo Robson, F. R. C. S. |
| Wm. B. Coley, M.D. | Karl G. Lennander, M.D. | W. L. Rodman, M.D. |
| W. M. L. Coplin, M.D. | Bransford Lewis, M.D. | Eugene A. Smith, M.D. |
| George W. Crile, M.D. | R. W. Lovett, M.D. | Harmon Smith, M.D. |
| Harvey Cushing, M.D. | W. G. MacDonald, M.D. | Wm. G. Spiller, M.D. |
| J. Chalmers Da Costa, M.D. | Edward Martin, M.D. | J. Bland Sutton, F. R. C. S. |
| John C. Da Costa, Jr., M.D. | Rudolph Matas, M.D. | Weller Van Hook, M.D. |
| E. B. Dench, M.D. | Chas. A. Mayo, M.D. | J. P. Warbasse, M.D. |
| F. X. Dercum, M.D. | Wm. J. Mayo, M.D. | F. C. Wood, M.D. |
| G. E. deSchweinitz, M.D. | Maj. Walter D. McCaw | George Woolsey, M.D. |
| David L. Edsall, M.D. | B. G. A. Moynihan, F. R. C. S. | Hugh H. Young, M.D. |

Kelly and Noble's Gynecology and Abdominal Surgery

Gynecology and Abdominal Surgery. Edited by HOWARD A. KELLY, M. D., Professor of Gynecology in Johns Hopkins University; and CHARLES P. NOBLE, M. D., Clinical Professor of Gynecology in the Woman's Medical College, Philadelphia. Two imperial octavo volumes of 900 pages each, containing 650 illustrations, mostly original. Per volume: Cloth, \$8.00 net; Half Morocco, \$9.50 net.

VOLUME I JUST READY—VOLUME II READY IN JULY

650 ILLUSTRATIONS BY BECKER AND BRÖDEL

In view of the intimate association of gynecology with abdominal surgery the editors have combined these two important subjects in one work. For this reason the work will be doubly valuable, for not only the gynecologist and general practitioner will find it an exhaustive treatise, but the surgeon also will find here the latest technic of the various abdominal operations. It possesses a number of valuable features not to be found in any other publication covering the same fields. It contains a chapter upon the bacteriology and one upon the pathology of gynecology, dealing fully with the scientific basis of gynecology. In no other work can this information, prepared by specialists, be found as separate chapters. There is a large chapter devoted entirely to *medical gynecology*, written especially for the physician engaged in general practice. Heretofore the general practitioner was compelled to search through an entire work in order to obtain the information desired. *Abdominal surgery* proper, as distinct from gynecology, is fully treated, embracing operations upon the stomach, upon the intestines, upon the liver and bile-ducts, upon the pancreas and spleen, upon the kidney, ureter, bladder, and the peritoneum. Special attention has been given to *modern technic*, and illustrations of the very highest order have been used to make clear the various steps of the operations. Indeed the illustrations are truly magnificent, being the work of *Mr. Hermann Becker* and *Mr. Max Brödel*, of the Johns Hopkins Hospital.

Bier's Hyperemic Treatment

By **WILLY MEYER, M. D.,** and **Prof. V. SCHMIEDEN**

Bier's Hyperemic Treatment in Surgery, Medicine, and the Specialties: A Manual of its Practical Application. By **WILLY MEYER, M. D.,** Professor of Surgery at the New York Post-Graduate Medical School and Hospital; and **PROF. DR. VICTOR SCHMIEDEN,** Assistant to Prof. Bier, University of Berlin, Germany. Octavo of 209 pages, with original illustrations. Cloth, \$3.00 net.

JUST READY—AN ENTIRELY NEW WORK

Bier's method of treating disease by inducing artificial hyperemia has assumed a place of first importance in modern therapeutics. It must not be supposed that this work is a translation: It is an entirely new and original work by Dr. Willy Meyer, the leading exponent of the treatment in this country, and Prof. Schmieden, Professor Bier's assistant at Berlin University. In the first part of the book the three methods of inducing hyperemia are described. In the second part are taken up the details of application.

Campbell's Surgical Anatomy

A Text-Book of Surgical Anatomy. By **WILLIAM FRANCIS CAMPBELL, M. D.,** Professor of Anatomy, Long Island College Hospital. Octavo of 675 pages, with 319 original illustrations. Cloth, \$5.00 net; Half Morocco, \$6.50 net.

JUST ISSUED—PRACTICAL ILLUSTRATIONS

The first aim in the preparation of this original work was to emphasize the practical. It is in the fullest sense an applied anatomy—an anatomy that will be of inestimable value to the surgeon because only those facts are discussed and only those structures and regions emphasized that have a peculiar interest to him. Dr. Campbell has treated his subject in a very systematic way, and illustrated his descriptions so graphically that the acquisition of anatomic relations has been rendered much more easy than has ever before been attempted.

Fowler's Treatise on Surgery

A Treatise on Surgery. By GEORGE R. FOWLER, M. D., Emeritus Professor of Surgery, New York Polyclinic. Two imperial octavos of 725 pages each, with 888 original text-illustrations and 4 colored plates. Per set: Cloth, \$15.00 net; Half Morocco, \$18.00 net.

RECENTLY ISSUED—IN TWO VOLUMES

Without doubt, Dr. Fowler's work is the most practical and complete surgery for its size ever published. *Every sentence tells its story*, either to recount a fact or give instruction as to treatment. The author especially emphasizes those injuries and surgical diseases that are of the greatest importance, not only because of their frequency, but also because of the difficulty of diagnosis and the special care demanded in their treatment. The text is elaborately illustrated with 888 entirely new and original illustrations, and every picture *actually shows* some surgical procedure, some step in the technic of an operation; every picture indicates *precisely how to do something*.

Rudolph Matas, M. D., *Professor of Surgery, Tulane University of Louisiana.*

"After a careful examination of this work I am glad to state that the completed text fully confirms the assurance I entertained: that this would prove a work of high order and distinct merit. These splendid volumes fully justify the repute of their author for earnestness, thoroughness, and learning."

Gant on Constipation and Intestinal Obstruction

Constipation and Intestinal Obstruction. By SAMUEL G. GANT, M. D., Professor of Diseases of the Rectum and Anus, New York Post-Graduate Medical School and Hospital. Octavo of 600 pages, with 235 original illustrations.

READY IN SEPTEMBER

This is a work for every practitioner and surgeon. The consideration given to the medical treatment of constipation is unusually extensive, and the chapter devoted to formulas will also be found invaluable to the practitioner. The descriptions of the operative procedures are concise, yet fully explicit. The 235 original pictures are as practical as the text.

Moynihan's Abdominal Operations

Abdominal Operations. By B. G. A. MOYNIHAN, M. S. (LOND.), F.R.C.S., England. Octavo, beautifully illustrated. Cloth, \$7.00 net; Half Morocco, \$8.50 net.

RECENTLY ISSUED—NEW (2d) EDITION

TWO LARGE EDITIONS IN ONE YEAR

It has been said of Mr. Moynihan that in describing details of operations he is at his best. The appearance of this, his latest work, therefore, will be widely welcomed by the medical profession, giving, as it does, in most clear and exact language, not only the actual *modus operandi* of the various abdominal operations, but also the preliminary technic of preparation and sterilization.

Edward Martin, M. D.

Professor of Clinical Surgery, University of Pennsylvania

"It is a wonderfully good book. He has achieved complete success in illustrating, both by words and pictures, the best technic of the abdominal operations now commonly performed."

Moynihan on Gall-stones

Gall-stones and their Surgical Treatment. By B. G. A. MOYNIHAN, M. S. (LOND.), F.R.C.S., Senior Assistant Surgeon, Leeds General Infirmary, England. Octavo of 458 pages, fully illustrated. Cloth, \$5.00 net; Half Morocco, \$6.50 net.

RECENTLY ISSUED—NEW (2d) EDITION

Mr. Moynihan, in revising his book, has made many additions to the text, so as to include the most recent advances. Especial attention has been given to a detailed description of the early symptoms in cholelithiasis, enabling a diagnosis to be made in the stage in which surgical treatment can be most safely adopted. A number of the illustrations are in color.

British Medical Journal

"He expresses his views with admirable clearness, and he supports them by a large number of clinical examples, which will be much prized by those who know the difficult problems and tasks which gall-stone surgery not infrequently presents."

Scudder's Fractures

WITH NOTES ON DISLOCATIONS

The Treatment of Fractures: with Notes on a few Common Dislocations. By CHARLES L. SCUDDER, M. D., Surgeon to the Massachusetts General Hospital, Boston. Octavo volume of 660 pages, with 854 illustrations. Polished Buckram, \$5.50 net; Half Morocco, \$7.00 net.

**JUST ISSUED—NEW (6th) EDITION, ENLARGED
OVER 25,000 COPIES**

Six large editions of this remarkable book is an undoubted indication of the popularity of Dr. Scudder's work. For this new edition numerous additions have been made throughout the text and a large number of new illustrations added, greatly enhancing the value of the work. The articles on Dislocations, illustrated in that practical manner which has made Dr. Scudder's book so useful, will be found extremely valuable.

Joseph D. Bryant, M.D., *Professor of the Principles and Practice of Surgery, University and Bellevue Hospital Medical College.*

"As a practical demonstration of the topic it is excellent, and as an example of bookmaking it is highly commendable."

Bickham's Operative Surgery

A Text-Book of Operative Surgery. By WARREN STONE BICKHAM, M. D., PHAR. M., of New York City. Octavo of 1000 pages, with 559 illustrations. Cloth, \$6.00 net; Half Morocco, \$7.50 net.

RECENTLY ISSUED—NEW (2d) EDITION

This absolutely new work completely covers the surgical anatomy and operative technic involved in the operations of general surgery. The practicability of the work is particularly emphasized in the 559 magnificent illustrations.

Boston Medical and Surgical Journal

"The book is a valuable contribution to the literature of operative surgery. It represents a vast amount of careful work and technical knowledge on the part of the author. For the surgeon in active practice or the instructor of surgery it is an unusually good review of the subject."

Gould's Operations on the Intestines and Stomach

The Technic of Operations upon the Intestines and Stomach. By ALFRED H. GOULD, M. D., of Boston, Massachusetts. Large octavo, with 190 original illustrations. Cloth, \$5.00 net; Half Morocco, \$6.50 net.

RECENTLY ISSUED

Dr. Gould's new work is the result of exhaustive experimentation, the technic of the operations described being simplified as far as possible by experiments on animals, thus leading to the development of many new features. The text is purposely concise, the technic being presented very clearly by the numerous practical illustrations.

New York State Journal of Medicine

"The illustrations are so good that one scarcely needs the text to elucidate the steps of the operations described. The work represents the best surgical knowledge and skill."

DaCosta's Modern Surgery

Modern Surgery. GENERAL AND OPERATIVE. By JOHN CHALMERS DACOSTA, M. D., Professor of the Principles of Surgery and of Clinical Surgery in the Jefferson Medical College, Philadelphia. Octavo of 1283 pages, with 872 illustrations. Cloth, \$5.50 net; Half Morocco, \$7.00 net.

RECENTLY ISSUED—THE NEW (5th) EDITION

For this new fifth edition the book has undergone a thorough and careful revision, and there have been added much new matter and nearly two hundred excellent and practical illustrations. Many new subjects and operations have been incorporated.

Boston Medical and Surgical Journal

"We commend the book, as we have previously commended it, to surgeons and to students as the most satisfactory one-volume contemporaneous treatise on surgery published in this country."

Schultze and Stewart's Topographic Anatomy

Atlas and Text-Book of Topographic and Applied Anatomy. By PROF. DR. O. SCHULTZE, of Würzburg. Edited, with additions, by GEORGE D. STEWART, M.D., Professor of Anatomy and Clinical Surgery, University and Bellevue Hospital Medical College, New York. Large quarto of 187 pages, with 25 colored figures on 22 colored lithographic plates, and 89 text-cuts, 60 in colors. Cloth, \$5.50 net.

RECENTLY ISSUED

It was Professor Schultze's special aim to produce a text-book and atlas not for the anatomist alone, but more particularly for the general practitioner. The value of the knowledge of topographic anatomy in bedside diagnosis is emphasized throughout the book.

Arthur Dean Bevan, M. D.

"I regard it as a very admirable work, for students especially, and find the plates and the text excellent."

Sobotta and McMurrich's Human Anatomy

Atlas and Text-Book of Human Anatomy. In Three Volumes. By J. SOBOTTA, M. D., of Würzburg. Edited, with additions, by J. PLAYFAIR McMURRICH, A. M., PH. D., Professor of Anatomy, University of Michigan. Three large quartos, each containing 250 pages of text and over 300 illustrations, mostly in colors. Per volume: Cloth, \$6.00 net.

VOLUME III JUST READY—COMPLETING THE WORK

Edward Martin, M.D., *University of Pennsylvania.*

"This is a piece of bookmaking which is truly admirable, with plates and text so well chosen and so clear that the work is most useful."

Eisendrath's Surgical Diagnosis

A Text-Book of Surgical Diagnosis. By DANIEL N. EISENDRATH, M. D., Adjunct Professor of Surgery in the College of Physicians and Surgeons, Chicago. Octavo of 775 pages, with 482 entirely new and original text-illustrations and some colored plates. Cloth, \$6.50 net; Half Morocco, \$8.00 net.

RECENTLY ISSUED

WITH 482 ORIGINAL ILLUSTRATIONS

Dr. Eisendrath takes up each disease and injury amenable to surgical treatment, and sets forth the means of correct diagnosis in a systematic and comprehensive way. Definite directions as to methods of examination are presented clearly and concisely, providing for all contingencies that might arise in any given case. Each one of the four hundred and eighty-two magnificent illustrations indicates precisely how to diagnose the condition considered.

Surgery, Gynecology, and Obstetrics

"The book is one which is well adapted to the uses of the practising surgeon who desires information concisely and accurately given."

Eisendrath's Clinical Anatomy

A Text-Book of Clinical Anatomy. By DANIEL N. EISENDRATH, A. B., M. D., Adjunct Professor of Surgery in the College of Physicians and Surgeons, Chicago. Octavo of 535 pages, with original illustrations. Cloth, \$5.00 net.

RECENTLY ISSUED—NEW (2d) EDITION

This new anatomy discusses the subject from the clinical standpoint. A portion of each chapter is devoted to the examination of the living through palpation and marking of surface outline of landmarks, etc. The illustrations are original.

Medical Record, New York

"A special recommendation for the figures is that they are mostly original and were made for the purpose in view. The sections of joints and trunks are those of formalized cadavers and are unimpeachable in accuracy."

International Text-Book of Surgery

Second Edition, Thoroughly Revised and Enlarged

The International Text-Book of Surgery. In two volumes. By American and British authors. Edited by J. COLLINS WARREN, M. D., LL. D., F. R. C. S. (Hon.), Professor of Surgery, Harvard Medical School; and A. PEARCE GOULD, M. S., F. R. C. S., of London, England.—Vol. I. *General and Operative Surgery*. Royal octavo, 975 pages, 461 illustrations, 9 full-page colored plates.—Vol. II. *Special or Regional Surgery*. Royal octavo, 1122 pages, 499 illustrations, and 8 full-page colored plates. Per volume: Cloth, \$5.00 net.

American TEXT-BOOK OF Surgery

FOURTH EDITION

American Text-Book of Surgery. Edited by W. W. KEEN, M. D., LL. D., HON. F. R. C. S., ENG. AND EDIN.; and J. WILLIAM WHITE, M. D., PH. D. Octavo, 1363 pages, 551 text-cuts and 39 colored and half-tone plates. Cloth, \$7.00 net.

Robson & Cammidge on Pancreas

The Pancreas: its Surgery and Pathology. By A. W. MAYO ROBSON, F. R. C. S., of Leeds, England; and P. J. CAMMIDGE, F. R. C. S., of London, England. Octavo of 546 pages, illustrated. Cloth, \$5.00 net.

JUST READY

This new work, upon one of the most widely discussed subjects of the times, represents the original investigations of these eminent authorities. It takes up Anatomy, Embryology, Histology, Physiology, Pathology, Symptomatology, and Injuries and Diseases, and there are special chapters on Chemical Pathology and Diabetes. The text is illustrated.

American Illustrated Dictionary Recently Issued New (4th) Edition

THE AMERICAN ILLUSTRATED MEDICAL DICTIONARY. With tables of Arteries, Muscles, Nerves, Veins, etc.; of Bacilli, Bacteria, etc.; Eponymic Tables of Diseases, Operations, Stains, Tests, etc. By W. A. NEWMAN DORLAND, M. D. Large octavo, 840 pages. Flexible leather, \$4.50 net; with thumb index, \$5.00 net.

Howard A. Kelly, M. D., *Professor of Gynecology, Johns Hopkins*
"Dr. Dorland's dictionary is admirable. It is so well gotten up and of such convenient size. No errors have been found in my use of it."

Golebiewski and Bailey's Accident Diseases

ATLAS AND EPITOME OF DISEASES CAUSED BY ACCIDENTS. By DR. ED. GOLEBIEWSKI, of Berlin. Edited, with additions, by PEARCE BAILEY, M. D. Cloth, \$4.00 net. *In Saunders' Hand-Atlas Series.*

Helferich and Bloodgood on Fractures

ATLAS AND EPITOME OF TRAUMATIC FRACTURES AND DISLOCATIONS. By PROF. DR. H. HELFERICH, of Greifswald, Prussia. Edited, with additions, by JOSEPH C. BLOODGOOD, M. D., Associate in Surgery, Johns Hopkins University, Baltimore. 216 colored figures on 64 lithographic plates, 190 text-cuts, and 353 pages of text. Cloth, \$3.00 net. *In Saunders' Atlas Series.*

Sultan and Coley on Abdominal Hernias

ATLAS AND EPITOME OF ABDOMINAL HERNIAS. By PR. DR. G. SULTAN, of Gottingen. Edited, with additions, by WM. B. Coley, M. D. Cloth, \$3.00 net. *In Saunders' Hand-Atlas Series.*

Warren's Surgical Pathology Second Edition

SURGICAL PATHOLOGY AND THERAPEUTICS. By J. COLLINS WARREN, M. D., LL. D., F. R. C. S. (HON.), Professor of Surgery, Harvard Medical School. Octavo, 873 pages, 136 illustrations. Cloth, \$5.00 net; Half Morocco, \$6.50 net.

Zuckerkindl and DaCosta's Surgery Second Edition

ATLAS AND EPITOME OF OPERATIVE SURGERY. By DR. O. ZUCKERKANDL, of Vienna. Edited, with additions, by J. CHALMERS DACOSTA, M. D., Professor of the Principles of Surgery and Clinical Surgery, Jefferson Medical College, Phila. 40 colored plates, 278 text-cuts, and 410 pages of text. Cloth, \$3.50 net. *In Saunders' Atlas Series.*

Lewis' Anatomy and Physiology for Nurses

Recently Issued

ANATOMY AND PHYSIOLOGY FOR NURSES. By LEROY LEWIS, M.D., Surgeon to and Lecturer on Anatomy and Physiology for Nurses at the Lewis Hospital, Bay City, Michigan. 12mo of 317 pages, with 146 illustrations. Cloth, \$1.75 net.

A demand for such a work as this, *treating the subjects from the nurse's point of view*, has long existed. Dr. Lewis has based the plan and scope of this work on the methods employed by him in teaching these branches, making the text unusually simple and clear.

"It is not in any sense rudimentary, but comprehensive in its treatment of the subjects in hand."—*Nurses Journal of the Pacific Coast*.

McClellan's Art Anatomy

Recently Issued

ANATOMY IN ITS RELATION TO ART. By GEORGE MCCLELLAN, M.D., Professor of Anatomy, Pennsylvania Academy of the Fine Arts. Quarto volume, 9 by 12½ inches, with 338 original drawings and photographs, and 260 pages of text. Dark blue vellum, \$10.00 net; Half Russia, \$12.50 net.

Senn on Tumors

Second Revised Edition

PATHOLOGY AND SURGICAL TREATMENT OF TUMORS. By NICHOLAS SENN, M.D., PH.D., LL.D., Professor of Surgery, Rush Medical College, Chicago. Handsome octavo, 718 pages, with 478 engravings, including 12 full-page colored plates. Cloth, \$5.00 net; Sheep or Half Morocco, \$6.50 net.

Macdonald's Diagnosis and Treatment

A CLINICAL TEXT-BOOK OF SURGICAL DIAGNOSIS AND TREATMENT. By J. W. MACDONALD, M.D. Edin., F.R.C.S. Edin., Professor Emeritus of the Practice of Surgery and of Clinical Surgery in Hamline University in Minneapolis, Minn. Octavo, 798 pages, handsomely illustrated. Cloth, \$5.00 net; Sheep or Half Morocco, \$6.50 net.

Preiswerk and Warren's Dentistry

Recently Issued

ATLAS AND EPITOME OF DENTISTRY. By PROF. G. PREISWERK, of Basil. Edited, with additions, by GEORGE W. WARREN, D.D.S., Professor of Operative Dentistry, Pennsylvania College of Dental Surgery, Philadelphia. With 44 lithographic plates, 152 text-cuts, and 375 pages of text. *In Saunders' Atlas Series*. Cloth, \$3.50 net.

"Nowhere in dental literature have we ever seen illustrations which can begin to compare with the exquisite colored plates produced in this volume."—*Dental Review*.

Haynes' Anatomy

A MANUAL OF ANATOMY. By IRVING S. HAYNES, M. D., Professor of Practical Anatomy, Cornell University Medical College. Octavo, 680 pages, with 42 diagrams and 134 full-page half-tones. Cloth, \$2.50 net.

American Pocket Dictionary

Fifth Revised Edition
Recently Issued

THE AMERICAN POCKET MEDICAL DICTIONARY. Edited by W. A. NEWMAN DORLAND, A. M., M. D., Assistant Obstetrician, Hospital of the University of Pennsylvania, etc. 578 pages. Full leather, limp, with gold edges, \$1.00 net; with patent thumb-index, \$1.25 net.

"I am struck at once with admiration at the compact size and attractive exterior. I can recommend it to our students without reserve."—JAMES W. HOLLAND, M. D., *Professor of Medical Chemistry and Toxicology, at the Jefferson Medical College, Philadelphia.*

Beck's Fractures

FRACTURES. By CARL BECK, M. D., Professor of Surgery, New York Post-graduate Medical School and Hospital. With an Appendix on the Practical Use of the Röntgen Rays. 335 pages, 170 illustrations. Cloth, \$3.50 net.

Barton and Wells' Medical Thesaurus

Recently
Issued

A THESAURUS OF MEDICAL WORDS AND PHRASES. By W. M. BARTON, A. M., M. D., Assistant to Professor of Materia Medica and Therapeutics, Georgetown University, Washington, D. C.; and WALTER A. WELLS, M. D., Demonstrator of Laryngology, Georgetown University, Washington, D. C. 12mo of 534 pages. Flexible leather, \$2.50 net; thumb index, \$3.00 net.

Stoney's Surgical Technic

Recently Issued
New (2d) Edition

BACTERIOLOGY AND SURGICAL TECHNIC FOR NURSES. By EMILY M. A. STONEY, Superintendent at the Carney Hospital, South Boston, Mass. Revised by FREDERIC R. GRIFFITH, M.D., Surgeon, of New York. 12mo, 278 pages, illustrated. \$1.50 net.

"These subjects are treated most accurately and up to date, without the superfluous reading which is so often employed. . . . Nurses will find this book of the greatest value"—*Trained Nurse and Hospital Review.*

Grant on Face, Mouth, and Jaws

A TEXT-BOOK OF THE SURGICAL PRINCIPLES AND SURGICAL DISEASES OF THE FACE, MOUTH, AND JAWS. For Dental Students. By H. HORACE GRANT, A. M., M. D., Professor of Surgery and of Clinical Surgery, Hospital College of Medicine. Octavo of 231 pages, with 68 illustrations. Cloth, \$2.50 net.

"The language of the book is simple and clear. . . . We recommend the work to those for whom it is intended."—*Philadelphia Medical Journal.*

Senn's SurgeryBased on the Author's
25 Years' Experience

PRACTICAL SURGERY. By N. SENN, M. D., PH. D., LL. D., Professor of Surgery in Rush Medical College. Octavo of 1133 pages, with 650 illustrations, many in colors. Cloth, \$6.00 net; Half Morocco, \$7.50 net. *Subscription.*

"A record of the matured opinions and practice of an accomplished and experienced surgeon."—*Annals of Surgery.*

Beck's Surgical Asepsis

A MANUAL OF SURGICAL ASEPSIS. By CARL BECK, M. D., Professor of Surgery, New York Post-graduate Medical School and Hospital. 306 pages; 65 text-illustrations and 12 full-page plates. Cloth, \$1.25 net.

Griffith's Hand-Book of Surgery

Recently Issued

A MANUAL OF SURGERY. By FREDERIC R. GRIFFITH, M. D., Surgeon to the Bellevue Dispensary, New York City. 12mo of 579 pages, with 417 illustrations. Flexible leather, \$2.00 net.

"Well adapted to the needs of the student and to the busy practitioner for a hasty review of important points in surgery."—*American Medicine.*

Keen's Addresses and Other Papers

Recently Issued

ADDRESSES AND OTHER PAPERS. Delivered by WILLIAM W. KEEN, M. D., LL. D., F. R. C. S. (Hon.), Professor of the Principles of Surgery and of Clinical Surgery, Jefferson Medical College, Philadelphia. Octavo volume of 441 pages, illustrated. Cloth, \$3.75 net.

Senn's Syllabus of Surgery

A SYLLABUS OF LECTURES ON THE PRACTICE OF SURGERY. Arranged in conformity with "American Text-Book of Surgery." By NICHOLAS SENN, M. D., PH. D., LL. D., Professor of Surgery, Rush Medical College, Chicago. Cloth, \$1.50 net.

"The author has evidently spared no pains in making his Syllabus thoroughly comprehensive, and has added new matter and alluded to the most recent authors and operations. Full references are also given to all requisite details of surgical anatomy and pathology."—*British Medical Journal.*

Keen on the Surgery of Typhoid

THE SURGICAL COMPLICATIONS AND SEQUELS OF TYPHOID FEVER. By WM. W. KEEN, M. D., LL. D., F. R. C. S. (Hon.), Professor of the Principles of Surgery and of Clinical Surgery, Jefferson Medical College, Philadelphia, etc. Octavo volume of 386 pages, illustrated. Cloth, \$3.00 net.

"Every surgical incident which can occur during or after typhoid fever is amply discussed and fully illustrated by cases. . . . The book will be useful both to the surgeon and physician."—*The Practitioner, London.*

Moore's Orthopedic Surgery

A MANUAL OF ORTHOPEDIC SURGERY. By JAMES E. MOORE, M. D., Professor of Clinical Surgery, University of Minnesota, College of Medicine and Surgery. Octavo of 356 pages, handsomely illustrated. Cloth, \$2.50 net.

"The book is eminently practical. It is a safe guide in the understanding and treatment of orthopedic cases. Should be owned by every surgeon and practitioner."—*Annals of Surgery.*

Fowler's Operating Room

Recently Issued
New (2d) Edition

THE OPERATING ROOM AND THE PATIENT. By RUSSELL S. FOWLER, M. D., Surgeon to the German Hospital, Brooklyn, New York. Octavo of 284 pages, illustrated. Cloth, \$2.00 net.

Dr. Fowler has written his book for surgeons, nurses assisting at an operation, internes, and all others whose duties bring them into the operating room. It contains explicit directions for the preparation of material, instruments needed, position of patient, etc., all beautifully illustrated.

Nancrede's Principles of Surgery

Recently Issued
New (2d) Edition

LECTURES ON THE PRINCIPLES OF SURGERY. By CHARLES B. NANCREDE, M. D., LL. D., Professor of Surgery and of Clinical Surgery, University of Michigan, Ann Arbor. Octavo, 407 pages, illustrated. Cloth, \$2.50 net.

"We can strongly recommend this book to all students and those who would see something of the scientific foundation upon which the art of surgery is built."—*Quarterly Medical Journal, Sheffield, England.*

Nancrede's Essentials of Anatomy.

Recently Issued
7th Edition

ESSENTIALS OF ANATOMY, including the Anatomy of the Viscera. By CHARLES B. NANCREDE, M. D., Professor of Surgery and of Clinical Surgery, University of Michigan, Ann Arbor. Crown octavo, 388 pages, 180 cuts. With an Appendix containing over 60 illustrations of the osteology of the body. Based on *Gray's Anatomy*. Cloth, \$1.00 net. *In Saunders' Question Compend.*

"The questions have been wisely selected, and the answers accurately and concisely given."—*University Medical Magazine.*

Martin's Essentials of Surgery. Seventh Revised Edition

ESSENTIALS OF SURGERY. Containing also Venereal Diseases, Surgical Landmarks, Minor and Operative Surgery, and a complete description, with illustrations, of the Handkerchief and Roller Bandages. By EDWARD MARTIN, A. M., M. D., Professor of Clinical Surgery, University of Pennsylvania, etc. Crown octavo, 338 pages, illustrated. With an Appendix on Antiseptic Surgery, etc. Cloth, \$1.00 net. *In Saunders' Question Compend.*

Martin's Essentials of Minor Surgery, Bandaging, and Venereal Diseases. Second Revised Edition

ESSENTIALS OF MINOR SURGERY, BANDAGING, AND VENEREAL DISEASES. By EDWARD MARTIN, A. M., M. D., Professor of Clinical Surgery, University of Pennsylvania, etc. Crown octavo, 166 pages, with 78 illustrations. Cloth, \$1.00 net. *In Saunders' Question Compend.*

1.D 83830

Saunders' Compends

10. ESSENTIALS OF GYNECOLOGY. 6th ed. With 57 illustrations.
By EDWIN B. CRAGIN, M.D. Revised by FRANK S. MATHEWS,
M.D.
11. ESSENTIALS OF DISEASES OF THE SKIN. 6th edition.
61 illustrations. By H. W. STELWAGON, M.D.
12. ESSENTIALS OF MINOR SURGERY, BANDAGING, AND
VENEREAL DISEASES. 2d ed. 78 illustrations. By
EDWARD MARTIN, M.D.
13. ESSENTIALS OF GENITO-URINARY AND VENEREAL DIS-
14. *QR46*
B3
1908 **268469**
Ball
15. **BIOLOGY**
LIBRARY
G
17. **UNIVERSITY OF CALIFORNIA LIBRARY**
19. **and 6 plates. By M. V. BALL, M.D. Revised by KARL M.**
VOGEL, M.D.
20. 21. ESSENTIALS OF NERVOUS DISEASES AND INSANITY.
4th ed. 53 illustrations. By JOHN C. SHAW, M. D. Revised
by SMITH ELY JELLIFFE, M. D.
22. ESSENTIALS OF DISEASES OF THE EAR. 3d ed., illus-
trated. By E. BALDWIN GLEASON, M. D.
23. ESSENTIALS OF HISTOLOGY. 3d ed. 106 illustrations. By
LOUIS LEROY, M. D.

W. B. SAUNDERS COMPANY, 925 Walnut St., Phila.

