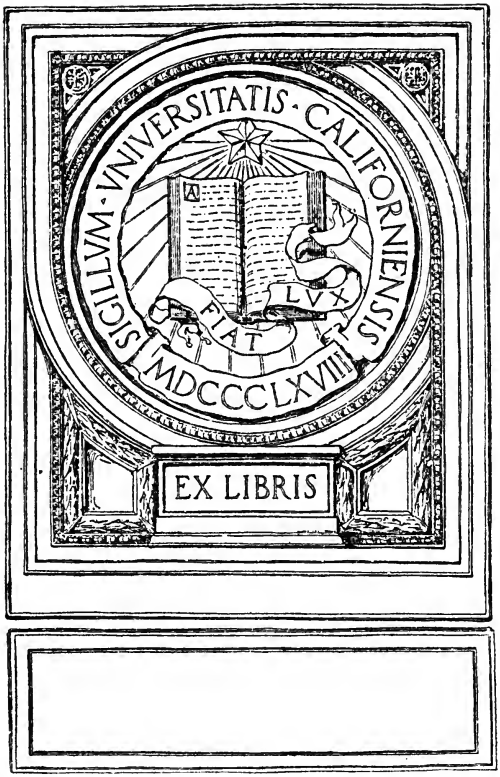
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EDWARD WILEY DUCKWALL
Director of the National Canners' Laboratory, Aspinwall, Pa.

CANNING *and* PRESERVING *of* FOOD PRODUCTS *with* BACTERIOLOGICAL TECHNIQUE

A PRACTICAL AND SCIENTIFIC HAND BOOK FOR MANUFACTURERS OF FOOD PRODUCTS, BACTERIOLOGISTS, CHEMISTS AND STUDENTS OF FOOD PROBLEMS. ALSO FOR PROCESSORS AND MANAGERS OF FOOD PRODUCT MANUFACTORIES

BY

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1905

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AFFECTIONATELY DEDICATED TO THE MEMORY OF

MY FATHER,

THOMAS DUCKWALL,

WHO WAS THE PIONEER CANNER AND MANUFACTURER

OF FOOD PRODUCTS IN OHIO.

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PREFACE

THERE are many valuable works written on the general subject of bacteriology, but nearly all such text-books apply the science either directly or indirectly to the field of medicine and surgery. Few authors have given any considerable space to the study of non-pathogenic bacteria, and very little attempt has been made to describe these species, beyond a few typical forms mentioned by the old authors.

While the pathogenic bacteria are occasionally found associated with the spoilage of food products, the non-pathogenic bacteria are far more common. Some of the pathogenic bacteria produce ptomaines and toxins in various food products, having gained entrance through contamination with diseased persons and animals, but these cases are extremely rare, owing to the rigid inspection of such products as are most liable to infection. Putrefactive bacteria are more commonly active agents in the production of ptomaines.

In this work we have endeavored to outline a course of study in bacteriology which will be particularly useful to the manufacturer and the student of food products. The causes of spoilage are defined, and the first volume is designed particularly to enable the student to gain a general knowledge of bacteriology which may be applied directly to solving problems of spoilage.

In the general plan have been introduced various well-known species of bacteria for comparative study, because the descriptions are given fully in nearly all text-books and the beginner will be better fitted for isolating and studying new species after he has completed a study of the well-known species.

There has been no attempt to classify or name many of the new species which were found associated with food spoilage, but the author has been satisfied to describe the action of these species on various food substances and has endeavored to ascertain the heat-resisting power of various spores.

The first volume of this work is designed especially to assist the student in a laboratory course in bacteriology applied to the manufacture of food products, particularly Canning and Preserving. The half-tones introduced as illustrations were made from photomicrographs taken by the author from specimens, stained and mounted, which were either isolated directly from spoiled food products or obtained through the courtesy of co-workers.

Several cultures of pathogenic bacteria were kindly furnished by Dr. F. G. Novy of the University of Michigan, and these were

stained, mounted and photomicrographed for illustrations in this book.

Chemical methods of analysis have been introduced for the benefit of the student. Some of these tests are very useful in the study of the products of fermentation and putrefaction. Other tests are given for the detection of adulterations and preservatives, all necessary for the study of food products.

In the beginning is a description of the microscope, lenses, apparatus, etc., employed in different parts of the work, together with a complete table of reagents used in the various preparations and chemical tests.

Then follows the description and classification of bacteria and methods of cultivating and staining. The method given for staining flagella is that employed by the author, with success. The various forms of decomposition are carefully studied, the last of which is the study of Putrefactive and Pathogenic organisms and the poisonous products elaborated by them.

Sterilization has been carefully studied in all its bearings, including Cleanly Methods of Manufacture and the Disposition of Waste Material.

Considerable space is devoted to the subject of Preservatives; their natural origin; their formation in canned goods during sterilization. This subject has been studied by the author and his assistants by feeding various animals stated daily amounts in their food for different periods of time. The feeding terms, weights and pathological effects on the internal organs, are faithfully described. Various theories of physicians and authorities of the harmful effect of these substances and their effect on processes of digestion have been analyzed. Many of these theories have been completely upset by actual tests and by force of argument. Throughout the whole subject, the author has been faithful to facts as he found them, and mere theories without the support of actual experiments and proofs have been criticised.

Whatever may be true as to the effect of at least two preservatives upon the human organism, no proofs of their harmfulness have been produced,—the preservatives studied in this connection are Salicylic and Benzoic Acids.

So far as I know, no person has come forward with the statement that he has ever been harmed in the least by eating them in table luxuries. The fact that thousands of tons of these two preservatives have been used by people who were under actual observation of the manufacturers, and the fact that no experiments with animals have produced pathological changes, seems to indicate that much of the hue and cry against them is not well founded.

Methods are given for extracting varous preservatives; also artificial sweeteners; also Miguel's table of Antiseptics and their value.

Artificial colors are not approved, and methods for detecting them are given.

The last part of this volume is taken up with the study of the canning of peas, tomatoes and corn. For twenty years the author has had practical experience in canning and preserving, having had charge of these departments for two of the largest houses in America, so that the practical and the scientific knowledge of his subject are brought closely together.

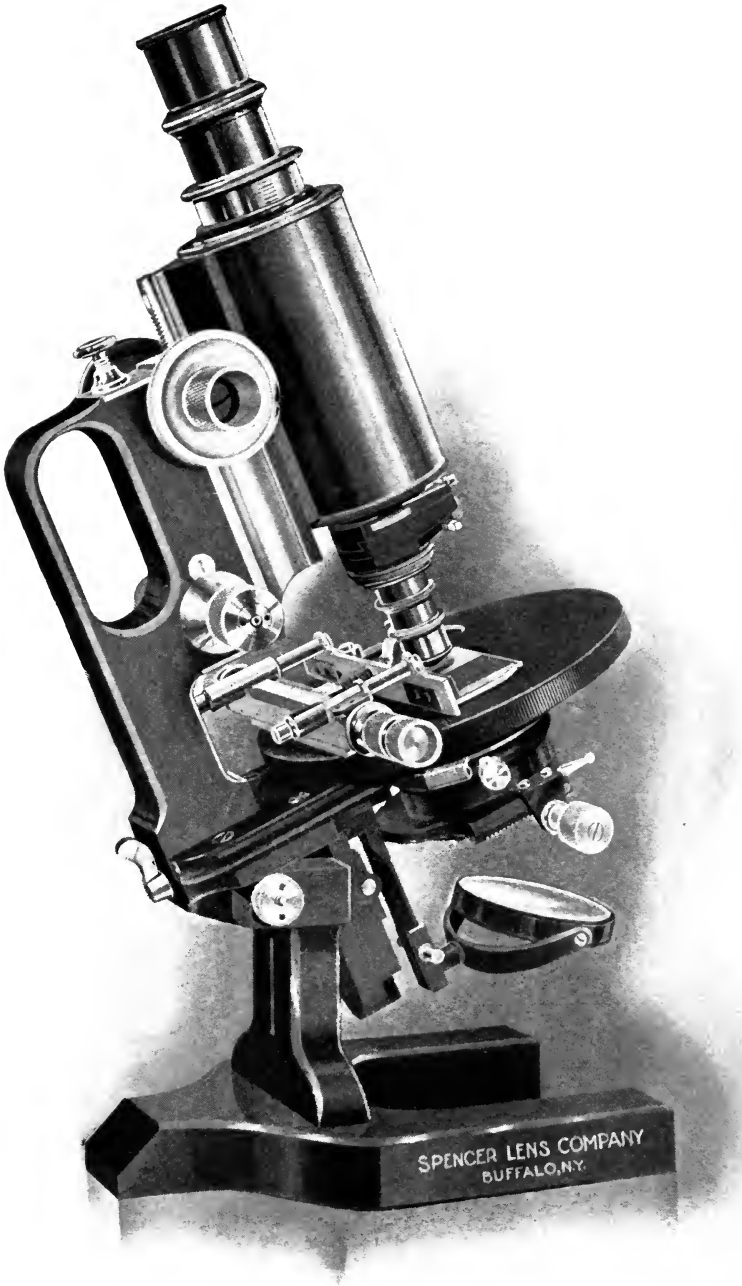
Considerable space has been devoted to the results obtained by bacteriological and chemical analyses of various spoilage cases together with suggestions made to correct imperfect methods.

Some laboratory work on tin plate is reproduced. The author has endeavored to show the action of fruits and vegetables on tin plate. Quantitative analyses of new tin plate were made, and the half-tones illustrate the imperfections as they appear on the ordinary plate used for canning purposes.

While this volume does not take in the whole list of canned and manufactured food products, in another valume the data at hand and the results obtained from the investigation conducted during the coming year will be published.

EDWARD W. DUCKWALL.

Aspinwall, Pa., Sept. 1, 1905.



MICROSCOPE

Canning and Preserving of Food Products with Bacteriological Technique

CHAPTER I.

The Laboratory and its Equipment

The Laboratory and Its Equipment. Apparatus Used in a Bacteriological and Food Laboratory. Description of Lenses. Table of Reagents.

THE MICROSCOPE.

THE MICROSCOPE is the most useful instrument needed for this work, and its selection is important in order to get the best possible combination of good qualities. No. 10 stand, objectives, and other attachments made by the Spencer Lens Company, of Buffalo, N. Y., give excellent satisfaction. The microscope requires careful attention; it should always be kept in a tidy condition, and it is quite necessary to know how to take care of the instrument in order that its delicate parts may not be injured. The stand shown in the cut has a handle, by means of which it may be carried, but most microscopes do not have this convenience and they must be lifted by the pillar below the level of the stage, and never by the fine adjustment tube or by the barrel. The lacquer of a microscope is injured by finger marks and should not be touched. Finger marks may be removed by breathing on the parts and gently rubbing with chamois skin. No chemical, such as alcohol or xylol, should be used to remove the marks, because it may remove the lacquer also. The microscope is provided with milled parts, which are the only parts to be handled when working.

The stage of the microscope should be kept clean and free from water. During the examination of live cultures of bacteria it may happen that some of the culture will get on the stage. It should be carefully removed with a rag moistened with bichloride of mercury solution.

OBJECTIVES.

The objectives are of two kinds, the dry and the oil immersion. The dry objectives should be kept clean with dry lens paper; they should never be allowed to touch oil, water or other substances. The oil immersion lens is very delicate and easily injured. It should

never be used dry, but always with a drop of cedar oil, and in the manipulation it should never be forced down against the glass slide or cover glass. Each evening after work the cedar oil should be removed from this objective by means of lens paper, moistened with xylol and dried with soft linen or lens paper. Alcohol or other chemicals should never be used for fear of dissolving the cement which holds the lens.

STANDS.

The selection of a stand is important, and while it may not be expensive it should be strong and firm in all its bearings. If photomicrographs are to be taken, the stability of the stand must be

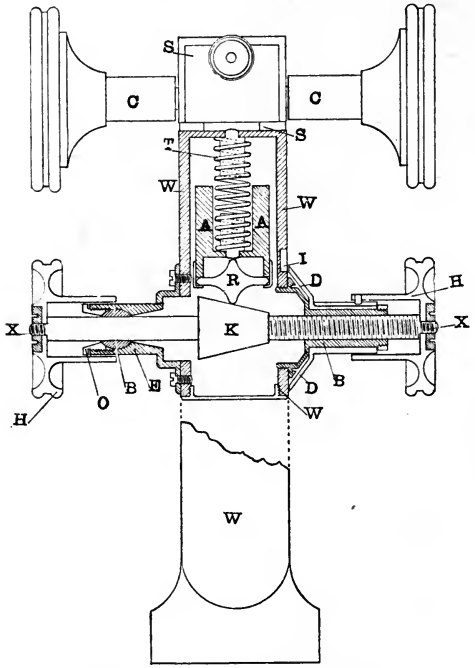


Fig. 1. Cone, fine adjustment

first-class. The *fine adjustment* is important. One cannot realize this until he begins to take photomicrographs. Many of the fine adjustment schemes are very unreliable and are easily jarred out of focus, particularly when the microscope is in a horizontal position. There is a new fine adjustment which is operated by a cone. This is so sensitive that the definitions of the reading drum, mark a vertical movement of the tube of 0.002 millimeters. This is a great advantage and is appreciated by the operator when making photomicrographs.

MECHANICAL STAGE.

A MECHANICAL STAGE is very useful and almost indispensable. The one shown in the cut gives an extended lateral move-

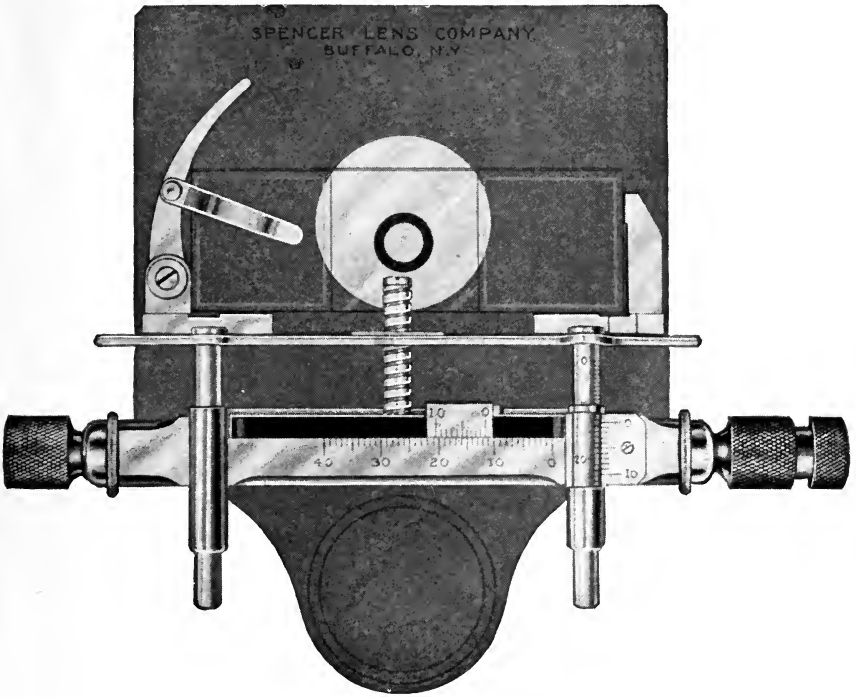


Fig. 2. Mechanical Stage

ment and the verniers are graded to read 0.1 m. m., and are placed closely together so as to be read at a glance. The mechanical stage has the advantage of holding in a steady position a particularly interesting view. It is also valuable in searching the field, which can be done systematically and with great precision.

OBJECTIVES.

OBJECTIVES are of two kinds, the dry and the oil. The dry objectives are seldom made in powers higher than 1/8 inch. For fine work, the apochromatic objectives are preferable to any others on account of their greatly superior correction of spherical and chromatic aberrations, which gives fine definition. The resolving power is also greater than the achromatic on account of their higher numerical aperture, but for ordinary work the achromatic objectives answer very well.

MICROMETER EYEPIECE.

For measuring objects under the objective it is advisable to use both the micrometer eyepiece and stage micrometer.

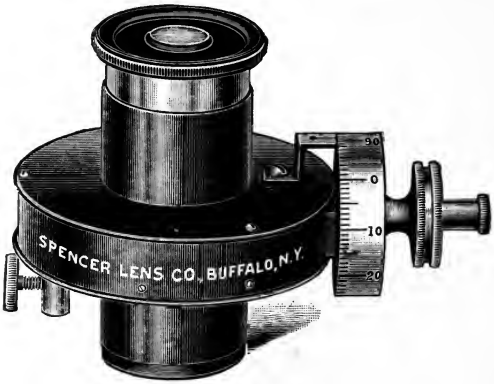


Fig. 3. Spencer Micrometer Eyepiece

DESCRIPTION OF LENSES.

Lenses are of two kinds, simple and compound; the former is generally a single lens. A simple microscope is therefore a simple lens. The rays of light come directly from the object to the eye and a *Virtual Image* is produced. (See figure 4, by Carpenter.) This illustrates the simple microscope.

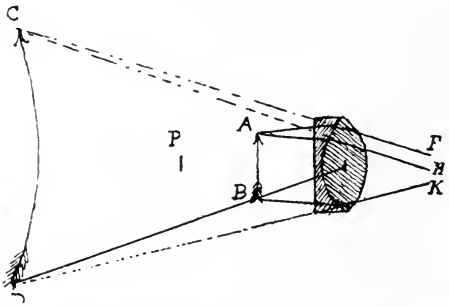


Fig. 4. Virtual Image, Simple Microscope (Carpenter)

The object is placed between the focus and the lens. This figure also illustrates the action of the eyepiece in the compound microscope. If the object is placed beyond the principal focus (p) a *real image* results, as is shown in figure 5. This illustrates the action of the objective in the compound microscope. In the compound miscroscope there are two sets of lenses, the eyepiece and the objective; the latter is nearest the object and produces a real

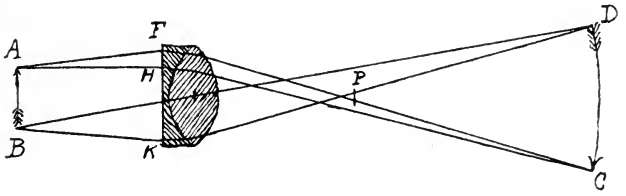


Fig. 5. Real Image (Carpenter)

image, as shown in figure. The image, however, is inverted and reversed; the light is inside the principal focus of the eyepiece and rays of light leave it if they come from a real object. The image

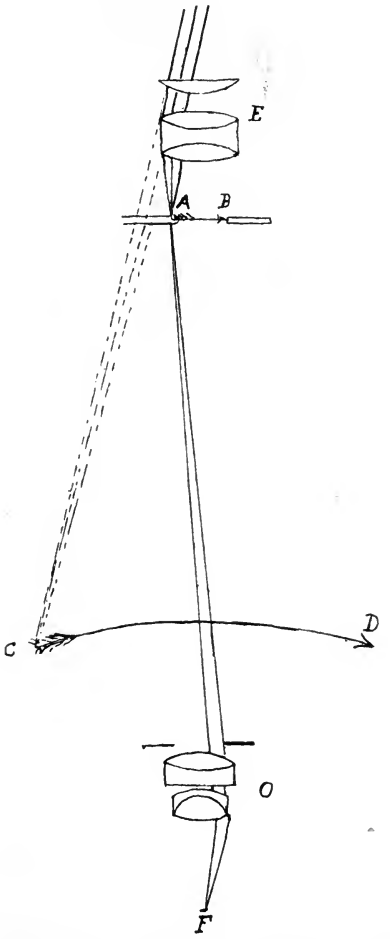


Fig. 6. Principle of a Compound Microscope (Carpenter)

F—Object in focus. O—Objective with diaphragm. AB—Real image of F, in the opening of the diaphragm; above this is a compensating ocular which magnifies the real image AB, this forming the virtual image CD

lying inside of the principal focus becomes magnified by the eyepiece (e) and the Virtual Image (c d) is produced.

Roger Bacon, an English monk, is said to have been the first man to recognize the peculiar properties of a lens, in 1276. The simple lens was used in the construction of spectacles. In the seventeenth century the microscope was perfected sufficiently to discern bacteria. Galileo made a compound microscope in the year 1610, and a cut of this microscope is shown, by Carpenter. This microscope contained a single lens for an objective and a single lens for an eyepiece. The rays from the single lens do not meet in the same plane and spherical aberration results. The rays of light are also decomposed in a simple lens, which acts as a prism; the violet is bent most and is brought to a focus at a different point from the red ray, which is bent the least. A fringe of colors results, and this is designated as "chromatic aberration". The spherical chromatic aberrations are corrected in the best instruments now made by means of diaphragms and stops and combinations of different kinds of glass. The chromatic aberration is fairly well corrected by the combination of the two glasses, Crown and Flint, and the objectives made from this combination are called Achromatic. Still, in the achromatic objective there is not absolute freedom from color. Even if some of the rays are neutralized, another will remain, and a certain amount of color will show in the image in the achromatic objective. This is designated as "secondary spectrum." In order to overcome this color, Abbe and Zeiss in 1889, prepared special kinds of glass, the so-called "borate" and "phosphate" glass, and this combination in objectives was designated as "apochromatic." Fluorite is also used in some apochromatics and the secondary spectrum is thus corrected. This correction is disturbed somewhat by the cover-glass, which refracts the peripheral rays as they enter the objective and they seem to come from a point nearer the objective than do the central rays. In order to overcome this the objectives are made "under-corrected," so that both points are focused at once. In order to get uniform results it is well to use cover-glasses of uniform thickness for the oil immersion objectives.

The most important part of the microscope, therefore, is the objective, and great care should be exercised in making a selection in order to get the best results. While good magnifying power is always desirable, it is of less value than the defining, resolving and penetrating power. A proper *magnifying power* is necessary, and it is customary to speak of an objective as a ⅔, ⅓, ⅙, ⅛, 1-12, etc. Some makers use these figures, others use letters, and some designate the magnifying power by millimeters. Every objective has what is called an initial magnification, and this initial magnification is multiplied by the magnifying power of the eye-

piece used. For instance, if the initial magnifying of a 1-12 objective is 125, when a No. 8 eyepiece is used, the magnification will be 1,000 diameters. Magnification is always meant as linear in scientific work, magnification being expressed as so many diameters. For instance, if a magnification is expressed as 1,000 diameters, the superficial area magnification would be 1,000,000.

THE DEFINING POWER.

It is difficult to secure perfect flatness of field. This is overcome to some extent by compensating eyepieces. Flatness of field is very essential in making photomicrographs. Probably the most important qualities of the microscope are its resolving and penetrating power. These are the qualities which show up the fine markings and delicate structures. These qualities have no reference to the magnifying power. The light which enters the objective has much to do with its resolving power.

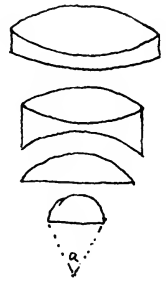


Fig. 7

Arrangement of Lenses in a 2 millimeter or $\frac{1}{12}$ Oil Immersion Objective (Carpenter)
A—Angle of Aperture

The light which enters the objective is included between the extreme rays, and an angle is formed by the extreme rays with the object in focus, and this angle is known as the "angle of aperture," shown by (a) in Fig. 7. Fig. 7 shows the system of lenses as they are arranged in the oil immersion objective. On account of the number of lenses employed, the light becomes very faint, and it is therefore necessary to admit as much light as possible. The improvement of an oil immersion lens over a dry objective is due to the fact that the light passing from the object in focus into the cover-glass is somewhat refracted, and unless it is collected again much of it is lost. Amici introduced the water immersion objective. This was an improvement over the dry objective from the fact that water would collect the rays of light fairly well, but in 1878 Stephenson suggested cedar oil, which has the same index of refraction as crown glass, and this has been in use ever since. A

high numerical aperture is then most valuable and objectives thus constructed are the most expensive.

The *penetrating power* of an objective depends upon its ability to show up objects in different planes. It is more highly perfected in the lower powers and there is still room for great improvement. In the examination of molds every worker is somewhat handicapped on account of improper penetrability.

ABBE CONDENSER.

A most important accessory to the microscope is the *Abbe condenser*. There are two special makes, the chromatic and the achromatic. The first is suitable for ordinary work, but for photomicrography the achromatic is almost indispensable. Fig. 8 shows a condenser of special construction, which can be swung out. Two diaphragms ordinarily go with it, one above and one below, and

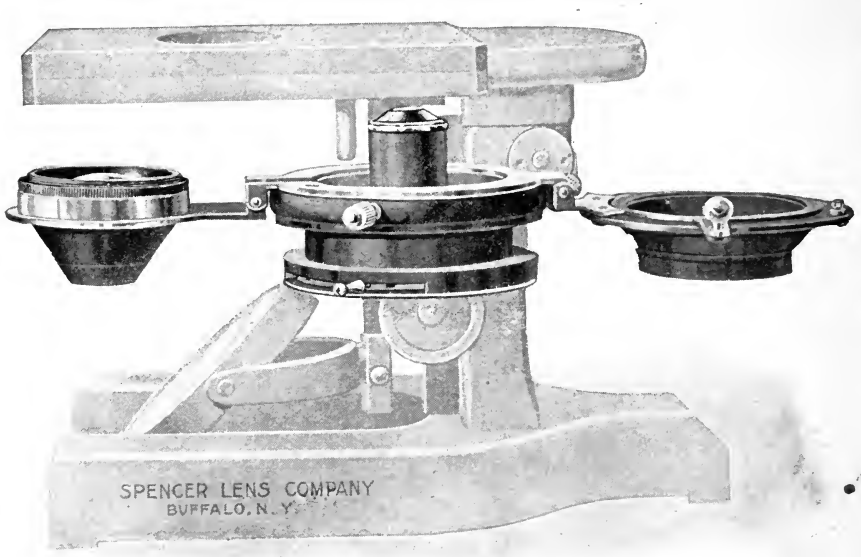


Fig. 8. Abbe Condenser

in addition to these there is a special arrangement made for holding the polariscope and other accessories for special illumination. The mirror under the Abbe condenser is plain on one side and concave on the other, the concave side being used to illuminate specimens in a living state, and the light is regulated with the iris diaphragm. The direct rays of the sun should never be used. Some writers prefer the light from a white cloud, but more uni-

form results are obtained from the Welsbach light. After one becomes accustomed to this light it becomes easier to make comparative study. For photomicrography the electric light is frequently employed, but the acetylene light is better in every respect, because the details are more clearly brought out.

PHOTOMICROGRAPHIC CAMERA.

This need not be an expensive apparatus. The one shown in Plate 2 is well suited for the work, because the camera and optical bench are solidly and conveniently held by a single support, giving them stability and perfect alignment without the risk of its disturbance.

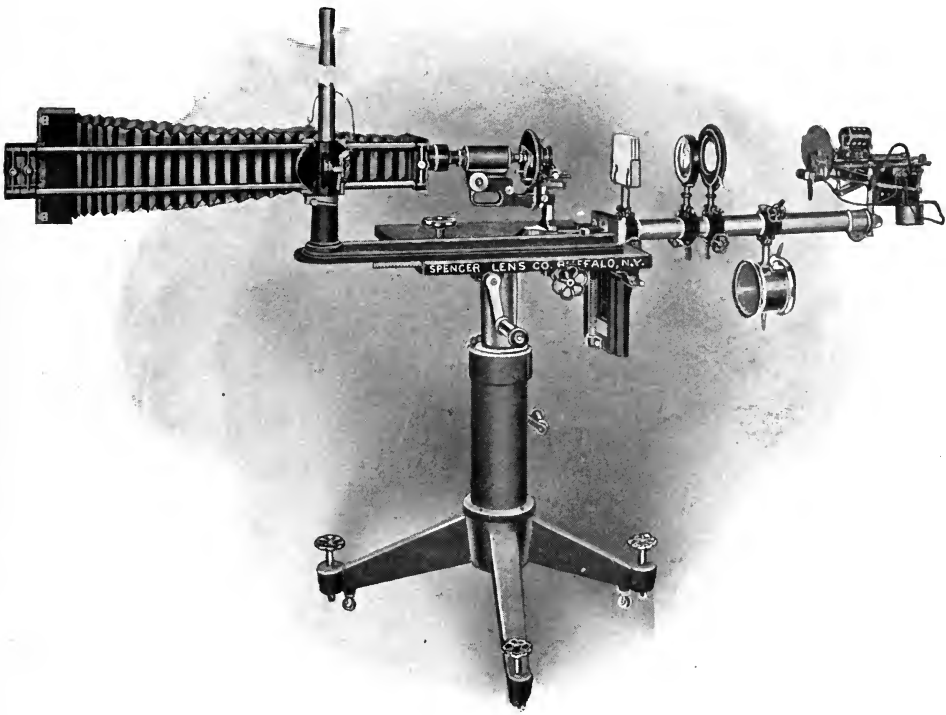


Plate 2. Photomicrographic Camera

In making good photomicrographs there are several points which must be carefully borne in mind in order to secure good results: First, a good slide preparation; second, a good microscope stand, provided with objectives and eyepieces, having the

qualities described under the head of "The Microscope;" a proper screen, acetylene light, and first-class isochromatic or orthochromatic plates. The development of the plates and the printing are all well understood by anyone familiar with photography.

THE INCUBATOR.

The one shown in the cut is admirably adapted for the cultiva-

Fig. 9. Incubator

tion of bacteria. To this must be attached a thermostat, which can be regulated, so that any desired temperature may be maintained constantly. This apparatus is very necessary in the cultivation of a great variety of bacteria and the usual temperature employed is 98°F., although for special organisms a room temperature is better. The incubator should be provided with a glass door

so that cultures may be observed without opening excepting when it is absolutely necessary. Heat is supplied by the Koch's safety burner, which turns off the gas supply automatically in case of accident. For testing canned goods to determine if the sterilizing process has been sufficient the incubator is almost indispensable.

THE AUTOCLAV.

This apparatus resembles in many respects the ordinary steam retort used in every cannery. A little water covers the bottom and steam is generated by means of a triple Bunsen burner, so that any desired temperature can be raised. As shown by the cut the

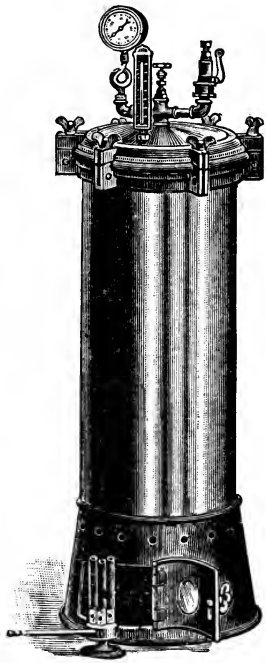


Fig. 10

top is arranged so that it can be clamped down, and a safety valve, thermometer and steam gauge are attached to the top so that they may be easily seen. This apparatus is used for sterilization of all culture media, and such media as are not injured by high temperatures are sterilized in one operation, where four or five operations are required in the laboratory not equipped with the autoclav. It affords an excellent means of determining the heat resisting power of various spores.

THE CENTRIFUGE.

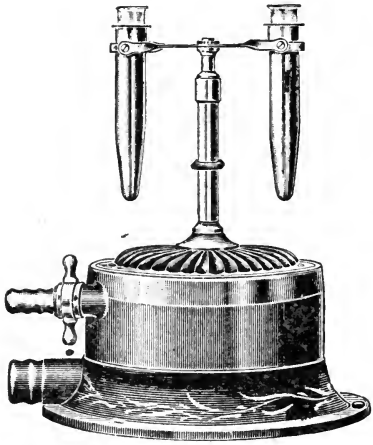


Fig. 11

This apparatus is used in the laboratory for various purposes. It is almost indispensable for breaking up emulsions formed by solvents in determining the presence of preservatives in food products. It is also useful in separating blood serum from the corpuscles. It is useful in precipitating the tubercule bacilli from sputum made into emulsions. It is used frequently in determining the presence of fat in milk, cream, cheese and other substances.

DISTILLING APPARATUS.

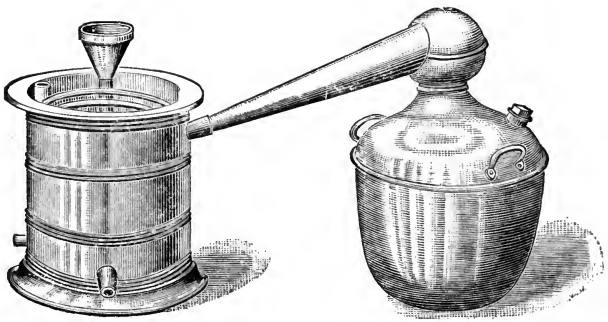


Fig. 12. Distilling Apparatus

This apparatus is necessary for distilling water and other substances where a clear solution is desired. It is made of heavy copper, is lined with movable head, and the condensing worm is made of pure block tin.

ANALYTICAL SCALES.

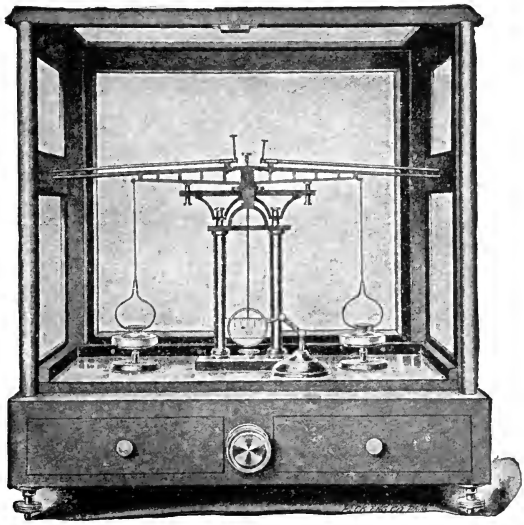


Fig. 13. Balances

These balances are graduated in 100 divisions for 1 m.m. and show the sensibility of 1/200 m. g. The beam and its hangings are of pure aluminum; the bearings are of agate with agate knives. These balances are used in fine analytical work, such as the determination of the amount of tin used on tin plate and the weighing of minute quantities of any chemical. Such a balance is indispensable in a well-equipped laboratory.

WATER BATH.

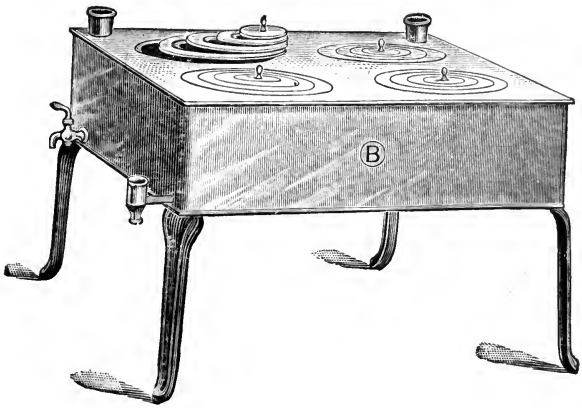


Fig. 14. Water Bath

This apparatus is employed for evaporating substances which

are affected by more intense heat. There is probably no apparatus in the laboratory which is employed more constantly than this.

PARAFFINE BATH.

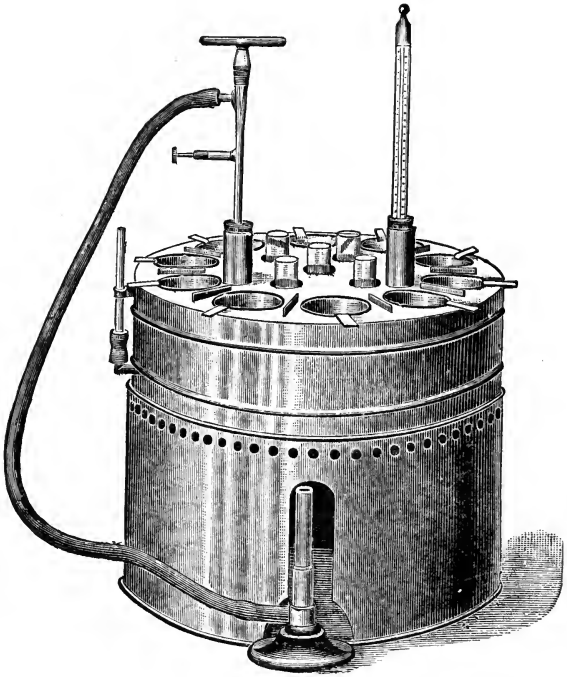


Fig. 15. Paraffine Bath

This bath is very useful for high temperatures and is the one employed for the conversion of saccharin into salicylic acid. It is very useful where temperatures running from 120°to 250°C. are required.

FORCEPS.

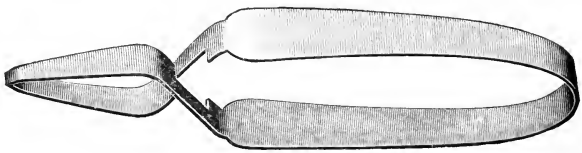


Fig. 16. Cornet Forceps

Fig. 17. Novy Forceps

The two cuts show the character of the forceps used generally in bacteriological work. The Novy forceps are convenient for handling cover-glasses, while the Cornet are large and firm and are so constructed that they will hold stains in fluid form on the cover-glass, so that they will not run down underneath nor onto the forceps.

THE MICROTOME.

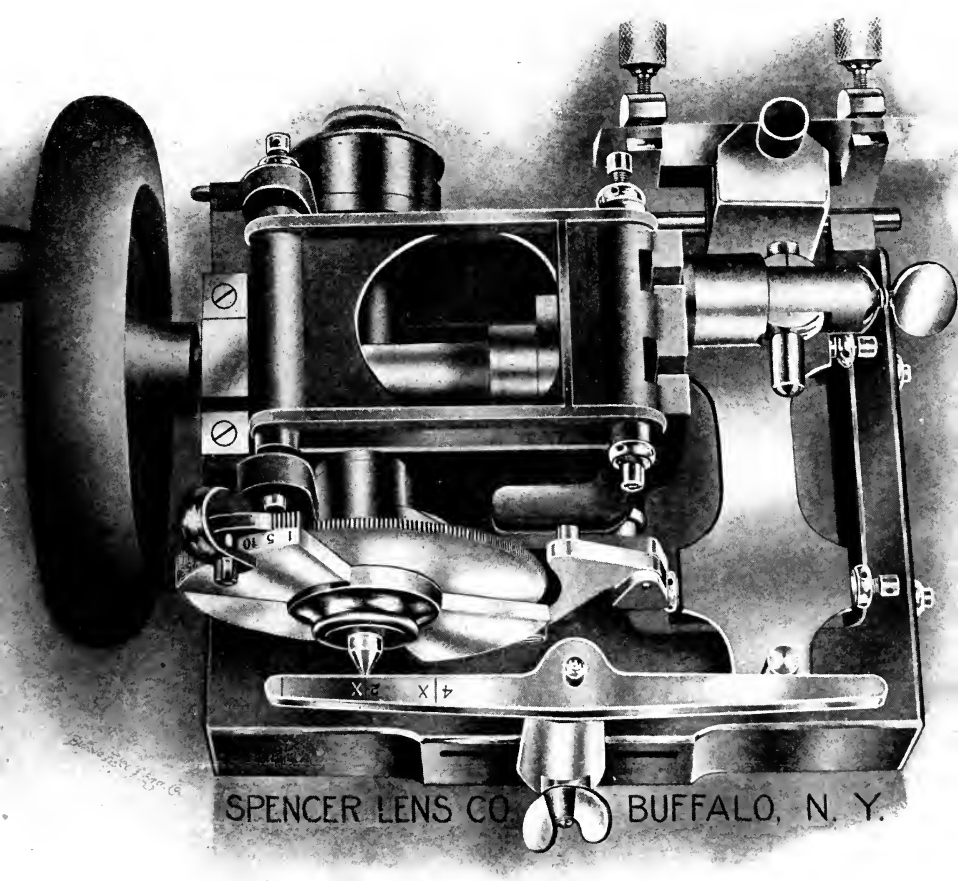


Plate 3. Microtome

This apparatus is used for making sections of the internal organs of such animals as Guinea pigs, rabbits, etc., shown in chapter on preservatives. The feed is controlled by an adjustable cam having a scale marked with a number of teeth, providing for any thickness of section from one up to twenty-five microns or more, and is absolutely reliable. The sections which are photographed in this volume were cut from one to two microns in thickness. For bacteriological and pathological work this apparatus is indispensable.

List of Apparatus and Chemicals

1 Agar apparatus.
1 Acetylene gas generator and burners.
1 Anaerobic culture apparatus.
1 Animal Holder.
3 Asbestos pads.
1 Aspirator.
1 Autoclav.
1 Nest of Beakers.
1 Blast lamp, bottles, various sizes, colored glass.
6 Boxes slides for microscope.
12 Boxes for test tube cultures.
2 Burettes, 50 c. c.
2 Burettes, 100 c. c.
2 Burette stands.
1 Buto-refractor.
2 Burners, Bunsen.
1 Centrifuge, Babcock's.
1 Chamberlain filter.
Cheesecloth.
1 Colony counter.
1 Copper soldering iron.
1 Corks, assorted sizes.
1 Cotton roll, absorbent.
2 ozs. Cover-glasses, No. 1.
1 Crucible.
1 Cylinder, 25 c. c. graduated.
1 Cylinder, 50 c. c. graduated.
1 Cylinder, 100 c. c. graduated.
1 Cylinder, 500 c. c. graduated.
1 Cylinder, 1000 c. c. graduated.
1 Dessicator.
1 Disinfecting jar.
1 Distilling apparatus.
4 Enameled pans, nested.
12 Esmarch dishes.
100 Filter paper, circles, 20 cm.
100 Filter paper, circles, 32 cm.
100 Filter paper, circles, 15 cm.
1 Square yard Flannel.
6 Flasks, Erlemeyer vacuum.
12 Flasks, Fiorentine, 500 c. c.
2 Funnels, large.
2 Funnels, 15 cm.
2 Forceps, Novy.
6 Forceps, Cornet.
2 Glass stiring rods.
6 Glass slides, hollow ground.
500 g. Glass tubing, 4mm. diam.
500 g. Glass tubing, 6mm. diam.
500 g. Glass tubing, 8mm. diam.
500 g. Glass tubing, 22mm. diam.
1 Hot pan.
1 Hydrogen generator, Kipp's.
1 Hydrogen sulphide generator.
1 Incubator, large, complete.
Labels, slide, jar and bottle.
1 Magnifier, simple lens.
1 Micrometer, cross-wire ocular.
1 Micrometer eyepiece.
1 Micrometer, stage.
1 Microscope, Spencer, No. 10 stand.
1 Mechanical stage, Spencer.
4 Eyepieces, 4, 6, 9, 12, compensating.
4 Objectives, 2-3, 1-6, 1-12, 1-16, apochromatic.
1 Abbe condenser, achromatic.

1 Triple nose piece.
1 Bull's eye, 3 in.
1 Microtome, Spencer.
1 Paraffine bath.
1 gross Petri-dishes, various sizes.
1 Photomicrograph camera, Spencer.
6 Pipettes, various sizes.
3 Platinum wires in glass rods.
1 Polariscope.
6 Porcelain dishes, nested.
3 Knives, peeling.
2 Retort stands.
Rubber stoppers, various sizes.
1 Sand bath.
1 Scissors, 14cm.
2 Separatory funnels.
1 Shears, tin.
1 Syringe, 5 c. c. inoculating.
200 Test tubes, 12 X 125 mm.
200 Test tubes, 15 X 150mm.
24 Test tubes, 20 X 150mm.
2 Test tube brushes for cleaning.
2 Thermometers, clinical.
1 Thermometer for retort.
1 Thermometer for paraffine bath.
2 Thermo-regulators.
2 Tripods.
1 Wash bottle.
6 Watch glasses, 5 cm.
1 Water bath.
2 Wax pencils, blue and yellow.
3 Wire baskets, medium.
6 Wire baskets, small.
6 Wire cages for animals.
3 Wire gauze.

CHEMICALS AND SUPPLIES

10 grams. Acetic acid, glacial.
100 grams Agar agar.
1000 grams Alcohol, absolute.
1000 grams Ammonium hydrate.
10 grams Benzoic acid.
100 grams Benzoate of sodium.
10 grams Boracic acid.
10 grams Borax.
100 grams Carbolic acid.
50 grams Celloidin.
3000 grams Chloroform.
100 grams Collodium.
10 grams Eosin.
3000 grams Ether.
50 grams Ferric tartrate.
50 grams Ferrous sulphate.
25 grams Fuchsine (not the acid).
250 grams Gelatin.
10 grams Gentian violet.
200 grams Glucose.
100 grams Glycerin.
50 grams Hematoxylin, Delifield's.
2500 grams Hydrochloric acid.
50 grams Iodin, resublimed.
30 grams Lactose.
50 grams Litmus.
Litmus paper, red and yellow.
100 grams Mercuric bichlorid.
15 grams Methylene blue.
1000 grams Nitric acid.
50 grams Cedar oil.
50 grams Clove oil.
3000 grams Paraffin.
200 grams Pepton, Witte's.
200 grams Potassium bichromate.
200 grams Potassium iodide.
5 grams Potassium ferrocyanide.
100 grams Pyrogallic acid.
100 grams Salicylic acid.
500 grams Salt.
200 grams Sealing wax.
200 grams Sodium carbonate.
500 grams Sodium hydrate.
500 grams Solder.
500 grams Steel filings.
2500 grams Sulphuric acid.
50 grams Tannic acid.
500 grams Tin foil.
50 grams Turpentine.
100 grams Vaseline.
500 grams Xylol.
3000 Zinc, granulated.
500 grams Zinc chlorid.
20 grams Canada balsam in tube.
100 pounds Carbide in drum.

TABLE OF REAGENTS. (After Leach.)

	NAME	FORMULA	DESIGNATION	Molecular Weight	Grams per Liter	REMARKS, PREPARATION, ETC.
1	Acid, acetic	$H C_2H_3O_2$	Glacial 99.5%	60		Sp. gr. 1.055
2	" carbolic	C_6H_5OH	Crystallized	64		
3	" hydrochloric	HCl	C. P. concentrated.	36.4	467.	Sp. gr. 1.20. 38.92% HCl.
4	" "		Dilute		265.	" " 1.12. 3 vols. No. 3 to 2 vols. H_2O. 23.63% HCl.
5	" "		10% solution			Sp. gr. 1.05. 1 vol. No. 3 to 8 vols. H_2O
6	" "		N-10 solution		3.64	Standardize against No. 114.
7	" "		Commercial, conc			Sp. gr. 1.20.
8	" nitric	HNO_3	C. P. concentrated	63	983.	" " 1.42. 69.2% HNO_3.
9	" "		Dilute		386.5	" " 1.20. 2 vols. No. 8 to 3 vols. H_1O. 32.2% HNO_3.
10	" "		Commercial, conc			Sp. gr. 1.42.
11	" oxalic	$CO_2H\ CO_2H+2H_2O$	Crystallized C. P	126		
12	" "		N-10 solution		6.3	Weigh freshly crystallized No. 11.
13	" phosphoric	H_3PO_4	U. S. P. sirupy			Sp. gr. 1.725.
14	" pyrogallic	$C_6H_3(OH)_3$	Crystallized	126		
15	" salicylic	$C_6H_4OH\ CO_2H$	"	138		
16	" sulphuric	H_2SO_4	C. P. concentrated	98	1843.	" " 1.8426. 100% H_2SO_4.
17	" "		Dilute		299.	" " 1.185. 25.27% H_2SO_4 1 vol. No. 16 to 5 vols. H_2O.
18	" "		10%		100.	For Reichert number.
19	" "		1.25%		12.5	For crude fiber.
20	" "		N-10		4.9	Standardize against No. 114.
21	" "		Commercial, conc			Sp. gr. 1.83 to 1.85 for Babcock process.
22	" tannic	$C_{14}H_{10}O_9$	Dry	322		
23	Alcohol, amyl	$C_5H_{11}OH$		88		" " 0.818. B. P. 132°C.
24	" ethyl	C_2H_5OH	Absolute	46		" " 0 7938. B. P 78.4°C.
25	Alum (ammonia)	$(NH_4)Al(SO_4)_2+12H_2O$	Crystallized	453		
26	Ammonia	NH_3	Concentrated 28%	17		" " 0.901.
27	"		Dilute 10% NH_3			" " 0.9597. 1 of NH_3 to 1.62 H_2O.
28	Ammonium carbonate	$(NH_4)_2CO_3H_2O$	Crystallized	114		

TABLE OF REAGENTS—Continued.

	NAME	FORMULA	DESIGNATION	Molecular Weight	Grams per Liter	REMARKS, PREPARATION, ETC.
29	Ammonium Carbonate		Solution		250.	
30	" chlorid	NH_4CL.	Dry salt	53.38		
31	" "		Solution		100.	
32	" molybdate	$(NH_4)_2MoO_4$	"			100 grams MoO_3 mixed with 400 c. c. cold H_2O and 80 c. c. ammonia No. 26. Filter and add filtrate to 300 c. c. HNO_3 No. 8 and 700 c. c. H_2O.
33	" sulphide	$(NH_4)_2S$	"			Saturate 3 vols. No. 27 with H_2S and add 2 vols. No. 27.
34	Animal charcoal		Dry			Pulverized bone charcoal
35	Asbestos fiber		Finely disintegrated			Washed in acid and alkali.
36	Barium chlorid	$BaCl_2H_2O$	Crystallized	244		
37	" "		Solution		100.	
38	Borax	$Na_2B_4O_7$ $10H_2O$	Crystallized	382		
39	Bromine	Br.	Liquid	80		
40	" water					Saturate H_2O with bromine, having a few drops bromine in excess in reagent bottle.
41	Calcium chlorid	$CaCl_2$ $_6H_2O$	Crystallized	219		
42	Canada Balsam					A solution in xylol.
43	Cane sugar					See sucrose.
44	Chloroform	$Ch._3$		119.1		
45	Chlor zinc iodide					
46	Distilled water	H_2O.		18		
47	Eosin	$C_{20}H_8Br_4O_5$				
48	Ether (ethyl)	$(C_2H_5)O$	U. S. P.	74		Sp. gr. 0.725 to 0.728. About 96% ether and 4% alcohol, with a little water.
49	Ferric chlorid	Fe_2Cl_6 $12H_2O$		541		
50	" "		Solution		100.	
51	Ferrous sulphate	Fe So_4 $_7H_2O$	Crystallized	278		
52	" "		Solution		100.	Prepare fresh for use.
53	" sulphide	FeS		88		
54	Formaldehyde		Solution			40% known as formalin.
55	Fuchsin	$C_{20}H_{19}N_3HCl$	Crystallized			

TABLE OF REAGENTS—Continued.

	NAME	FORMULA	DESIGNATION	Molecular Weight	Grams per Liter	REMARKS, PREPARATION, ETC.
56	Gelatin					
57	Hydrochloric acid;					
58	ferric chlorid reagent					Concentrated commercial HCl, with 2.5 cc. No. 50 per liter.
59	Hydrogen dioxid	H_2O_2		34		
60	Hydrogen sulphide	H_2S	Gas	34		Treat FeS with dilute H_2So_4.
61	Indol	C_8H_7N	Crystals	234		
62	Iodine	I	Resublimed	126		
63	"		N-10 solution		12.66	Triturate I in mortar with 18 grams KI and small proportions H_2O till all is dissolved. Dilute to 1 liter, standardizing against Sodium thiosulphate N-10 solution.
64	" in potassium iodide		Solution			2 grams crystallized KI dissolved in 100 c. c. H_2O. Saturate solution with I.
65	Lactose	$C_{12}H_{22}O_{11}H_2O$	Dry powder	360		
66	Lead acetate	$Pb(C_2H_3O_2)_2\ 3H_2O$	Crystallized	379		
67	" subacetate		Use U. S. P. solution			
68	Lime water					Saturate H_2O with $Ca(OH)_2$.
69	Litmus		Dry color			
70	"		Tincture			Digest 1 part litmus in 6 parts H_2O. Filter and divide into two parts. Slightly acidify 1 part by glass rod dipped in H_2So_4 (dil.) to faint red. Add other or blue portion of filtrate.
71	Litmus paper					Moisten filter paper in No. 70.
72	Magnesium oxid	MgO		40		
73	Manganese dioxid	MnO_2	Pulverized	87		
74	Mercury	Hg		200		
75	Mercuric chlorid	$HgCl_2$		271		
76	" "		Saturated solution			Saturate H_2O with $HgCl_2$.
77	" "		Dilute		50.	
78	Methyl blue					
79	" orange		Dry color			

TABLE OF REAGENTS—Continued.

	NAME	FORMULA	DESIGNATION	Molecular Weight	Grams per Liter	REMARKS, PREPARATION, ETC.
80	Methyl orange		Indicator solution		1	In H_2O. Add H_2SO_4 until sol. turns red, filter.
81	Milk-sugar					
82	Paraffin					
83	Phenol					
84	Phenolphthalein	$C_{20}H_{14}O_4$	Dry powder	318		
85	"		Indicator solution		10	Dissolve in 50% alcohol.
86	Potassium bichromate	$K_2Cr_2O_7$	Dry salt	295		
87	" carbonate	K_2CO_3	" "	138		
88	" ferricyanide	$K_3Fe(CN)_6$	" "	329		
89	" "		Solution		50	Prepare freshly for use.
90	" ferrocyanide	$K_4Fe(CN)_6 3.H_2O$	Crystallized	422		
91	" "		Solution		50	
92	" hydroxid	KOH	Dry substance	56		
93	" "		Solution		500	
94	" "		"		1000	For CO_2 determination.
95	" iodide	KI	Crystallized	166		
96	" "		Solution		100	
97	" nitrate	KNO_3	Crystallized	101		
98	" permanganate	$KMnO_4$	"	158		
99	" "		N-10 solution		3.16	Adjust strength of solution by repeated trial.
100	" sulphate	K_2SO_4	Crystallized	174		
101	Resorcin	$C_6H_4(OH)_2$	"	110		
102	"		Solution		100	
103	Schiff's reagent					Dissolve 1 gram of fuchsin in H_2O, add a mixture of 20 c c. $NaHSO_3$ solution No. and 10 c. c. HCL No. 8. Make up to 1 liter.
104	Schultze's reagent					
105	Silver nitrate	$AgNO_3$	Crystallized	170		
106	" "		Solution		25	
107	" "		N-10 solution		16.955	
108	Sodium	Na	Metal	23		Keep under petroleum.
109	" bisulphite	$NaHsO_3H_2O$	Crystallized	104		

TABLE OF REAGENTS—Continued.

	NAME	FORMULA	DESIGNATION	Molecular Weight	Grams per Liter	REMARKS, PREPARATION, ETC.
110	Sodium bisulphite		Solution			Saturated.
111	" carbonate	Na_2CO_3	Dry Substance	106		
112	" hydroxid	NaOH	" "	40		
113	" "		Solution		500	
114	" "		N-10 solution		4	Adjust strength of solution by repeated trial. Standardizing against No. 12.
115	Stannous chlorid	$SnCl_2 2H_2O$	Solution			Saturate conc. HCl with tin, dilute with an equal vol. of H_2O and from time to time add slight excess of acid. Keep pieces of tin in reagent bottle.
116	Starch, arrowroot					
117	"		Indicator solution		10	Mix into thin paste with cold H_2O. Pour into 100 times its weight of boiling H_2O. Boil, settle and use supernatant liquid. Use fresh solution.
118	Sucrose	$C_{12}H_{22}O_{11}$	Crystallized			Powdered rock candy.
119	Tannin					See acid, tannic.
120	Tin	Sn	Granulated	118		
211	Tumeric		Dry Powder			
122	" paper					Make strong alcoholic solution of No. 121, dip strips of paper therein, and dry.
123	Xylol	C_8H_{10}				As a medium for Canada balsam.
124	Zinc	Zn	Granulated	65.1		
125	" chloriodide					See No. 45.

CHAPTER II.

Bacteria—Description and Classification

Spore Formation. Nature of Bacteria. Influence of Electricity on Bacteria. Influence of Various Temperatures. Influence of Light. Motility. Chromogenic Bacteria. Bacterial Products. Slime, Ropiness, Etc.

Bacteria belong to the lower vegetable kingdom and are not properly named germs or microbes, which terms embrace a larger meaning including animalcules and lower insect life. In 1683 Leeuwenhoek made the discovery of bacteria while examining the scraping of the teeth, and for nearly two hundred years bacteria were thought to be animal life. In 1875, several investigators, Cohn, Naegeli and others, settled the fact that they belonged to the vegetable kingdom, principally on account of their resemblance to the algae in their manner of reproduction, growth and multiplication. The distinction between these low vegetable forms and the lowest forms of animal life is not so easily determined as we might suppose, for the reason that there are so many unknown facts concerning both, which science up to this time has not been able to make clear. Although the microscope has been brought to a degree of perfection hardly to be improved, there are still forms of life which are beyond its power. It was thought until recently that bacteria were the smallest forms of life, but the researches of Dr. Koch and others have brought to light that there are still smaller organisms which are the probable causes of maladies such as Foot and Mouth disease, Horse Sickness and Rinderpest.

However, there are so many characteristics of plant life in bacteria, that they are properly assigned to the vegetable kingdom. They resemble in many ways the higher forms of microscopical plant life, viz.: molds and yeasts which are classed as fungi, but in size they are very much smaller. Bacteria multiply by fission or division, from which characteristic they are termed Schizomycetes. The division takes place by a lengthening, when a constriction takes place thus:

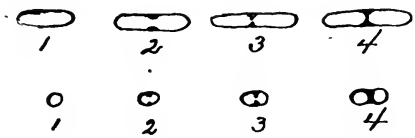


Fig. 18

The yeasts multiply by budding as also do some of the molds under certain conditions. The yeasts are termed Blastomycetes. The molds naturally grow in a thread-like manner, spreading out and sending up fine hair-like tufts or hyphae, hence they are termed Hyhomycetes. The classification of bacteria is not complete. There are various forms which are taken as types, but changes often take place due to the character of the substances in which they are found, which cause one class to appear very like another class. The classification as we have it is based upon the form, size, motility, manner of dividing, formation of spores, the presence of flagella or hair-like propellers, their ability to resist high temperatures, the enzymes or products resulting from their growth, the colors formed by certain kinds, the flavor produced by others, their manner of growth on certain artificial media or food specially prepared for them and many other ways, but first they can be divided into three classes with reference to external appearance.

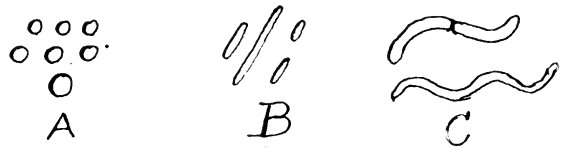


Fig. 19

A—Micrococcus or single round cells
B—Bacillus or rod form.
C - Spirillum or spiral form.

A very short bacillus is often termed a bacterium. The spirilla or curved forms are often joined together to form a spiral and were called vibrios in Pasteur's time. There are very great differences in sizes of bacilli, some being quite short and thick, others very long and delicate, and vice versa. Some have square ends, others are rounded.

There are many different kinds of bacteria which resemble each other in all that the microscope will reveal, and their identification depends upon their behavior under various conditions. It is a fact that the identification of very few bacteria can be determined by the microscope alone, but after observing a certain form in many conditions, its identity may be pretty clearly determined. Changes in temperature while growing; cultivations in acid or alkaline media; cultivations in fluid and on solid media may give rise to varied shapes, colors and products, which may be noted and the character of the organism established.

The action of chemicals, dyes, salt and sugar solutions may cause what is known as plasmolysis, which is a shrinking of the cell membrane and the granulation of the protoplasm or contents of the cell. Sometimes the cell membrane disappears so that the bacillus appears as a row of little round balls.

One fact has been established which is interesting to the student of evolution, i. e., one kind of bacillus never develops into another kind. Like springs from like, just as in the higher forms of life. If there are hybrids among them it has not been proven, but there is some evidence of this in disease organisms, particularly in mammal and avian tubercule bacilli. Bacteria are known to change completely in some respects, but their identity is not lost. We have seen certain species which ordinarily do not liquefy gelatin, suddenly divide into two classes, one of which will ever afterward liquefy gelatin, and vice versa. Proteus Zenkeri is probably a type of this species.

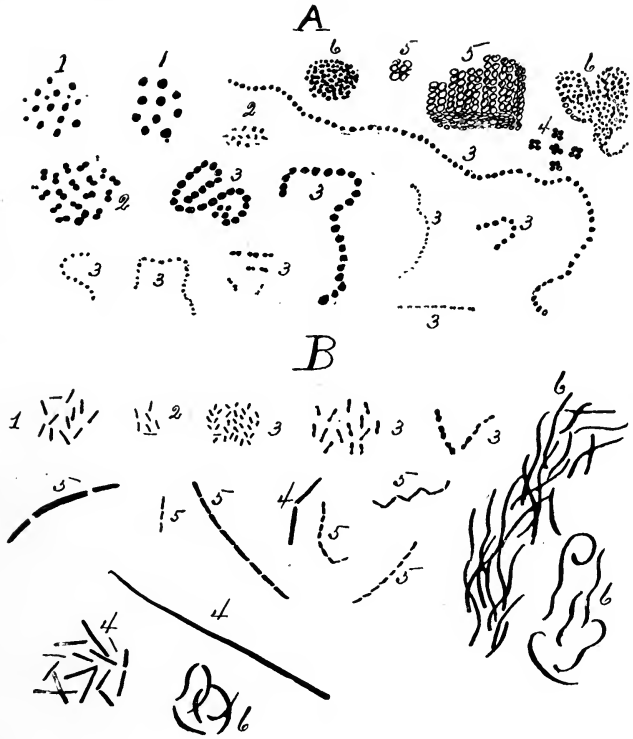


Fig. 20

Illustrations of the manifold variety in size and form of different bacteria. Except A4 and A5 all the above illustrations are representations of equally magnified bacteria from a single drop of putrescent blood (after P. Baumgarten) x 950.

A-1 Cocci (micrococcus) of various sizes.
2 Diplococci of various sizes.
3 Streptococci of various sizes.
4 Micrococcus tetragonus (from pure culture) magnified 950.
5 Sarcina ventriculi, magnified 700
6 Staphylococci.
B-1, 2, 4 Separate long rods of various lengths and breadths.
3 Short rods, partly of biscuit form.
5 Chains composed of either short or long rods.
6 Long threads.

The illustration in Fig. 20 gives a good idea of the appearance of different types as they appear under the microscope.

The manner of *vegetation* or *multiplication* is as follows:

The *Coccus* vegetates by becoming longer, constriction follows and finally complete separation.

When they remain united in twos they are named *Diplococci.*

When they divide in two directions to make fours, they are named *Tetragoni.*

When they remain united forming chains they are named *Streptococci.*

When they form bunches resembling grapes they are named *Staphylococci.*

When they divide in three directions making eight cells and remain united in bundles they are named *Sarcina.*

The rod forms may lengthen, divide and separate, or may form pairs or chains resembling sausages. These chains are easily broken up by agitation so that they appear more frequently in the field of view under the microscope in all three forms.

LIFE HISTORY OF BACTERIA.

Bacteria are present almost everywhere, in the ground, in the air, clinging to dust and floating matter, and in water. They find their way into living bodies and plants and are most numerous where there is decomposition of organic matter. The air in mid-ocean is free from them, because all suspended particles are deposited in the water and the air is washed until it is pure. The air at high altitudes is almost free from bacteria and the soil at the depth of twelve feet also. Water from artesian wells is almost free, and whatever contamination it has is due to the deposit of germ life from the surface. The air that is exhaled from the lungs is free; no matter how many thousands of bacteria are inhaled, they are caught by the hairs and mucous in the air passages and cast out or eventually destroyed, except where disease is contracted. The origin of bacteria is not known, but is shrouded in the mystery of creation. There is evidence that new species are created, although it cannot be stated as a positive fact. New diseases make their appearance and cases of spoilage in food products occur which seem to be new. The great majority of bacteria are harmless to man, indeed are very necessary and indispensable as decomposing agents of dead organic matter. Through their instrumentality, obnoxious accumulations are reduced to elementary forms capable of building up new life, both animal and vegetable.

There is another class called *pathogenic bacteria* which are instrumental in the destruction of living animals, including man. This class is to the bacterial flora what the poisonous plants and weeds are to the higher forms in the vegetable kingdom, and like these

are in the minority. Bacteria, as we have stated, are almost universally distributed, but are not always in the full vegetating form as we see them under the microscope. They become dried up, or in spore form are wafted through the air or are carried by water until they are lodged upon certain kinds of organic material which furnish them the necessary elements for growth, which is termed vegetation. This is a multiplication which continues until certain conditions arise, such as change of temperature or chemical composition, due to their own action or the products formed by other kinds of bacteria vegetating at the same time with them, or by conditions arising from natural causes, when they either perish or pass into a resting or dormant state. The resting state is characterized by a drying up of the cell membrane or the formation of spores. While it is probable that nearly all bacteria give rise to spores of some kind, this has not been demonstrated as a fact, because the conditions under which we cultivate them for study and observation are not always as favorable as the conditions under which they grow naturally; then, again, the extreme minuteness of many forms prevents the close examination necessary to establish a complete life history. There is evidence that spore formation may go on in a field far beyond the power of our best microscopes. The phenomenon of *spore formation* is observed, however, in the life history of a large number of bacteria, and the formation and liberation of spores in many cases can be watched with interest in the hanging drop cultures. The formation of spores is not always due to the causes assigned above, viz., the exhausting of the food supply, etc., for it frequently occurs when the nourishment is most favorable for natural vegetation or multiplication. We must make a clear distinction between spore formation and vegetation, the spores correspond to the seed in higher plant life and are formed for the perpetuation of the species, while the vegetation is a multiplication, not by seed formation but by division, and may go on almost indefinitely, if the bacteria are constantly transplanted into fresh nutrient material. (There are exceptions to this however.) The pathogenic bacteria do not naturally show spore formation, because the living body supplies them constantly with fresh material for multiplication. Some of the pathogenic bacteria when grown artificially in the laboratory on nutrient media, do give rise to spores, showing their relationship to the ordinary non-pathogenic bacteria. Anthrax and Tetanus are examples of this kind.

Fig. 21. Sporulation

a—First stage showing granules. b—Incomplete spore. c—Developed spore. (After Novy.)

Nearly all text-books speak of two kinds of spore formation, viz., *endospores* and *arthrospores*, from a theory advanced by De Bary. The endospore is always formed within the cell. The arthrospore formation is supposed to be a complete thickening of the

Plate 4. Anaerobic Pea Bacillus

Photomicrograph of the Spore-bearing rods of the Anaerobic Pea Bacillus found in a can of swelled peas The spores are terminal and greatly resemble Bacillus tetanus. Magnified 1,000 diameters.

cell membrane which contracts so that the cell thus becomes a spore. The arthrospore theory, however, is not well founded because the actual observance of this phenomenon is wanting. We are only certain of endospore formation. The formation of spores is thus observed: The whole bacillus is first seen as a colorless, homogeneous cell, showing no bright spots. When it advances to the state of spore formation, fine granules can be detected scattered throughout the cell, some very small, others larger and irregular, one bright spot continues to grow larger and brighter and the other granules may probably be absorbed by it—the bright spot, at first irregular, now begins to assume definite shape, either round or ellipsoidal, with a dark line forming around it which seems to grow thicker, forming a wall which seems to enclose all the contents or protoplasm of the cell, or the protoplasm may go to build up the spore wall, which is probably the case. The old cell is now merely a shell containing the spore and may soften and disappear in the surrounding fluid, leaving the bright spore in a free state. In some cases the cell remains together with the spore and may not dissolve. This complete process may occupy several days, but is often accomplished in a much shorter time.

Ordinarily one spore develops within a single bacillus, but A. Koch has mentioned that Bacillus Inflatus has two; this is improbable. Not every bacillus gives rise to spores—it sometimes is observed that a whole chain of bacilli will be seen with spores excepting one or two which seem barren.

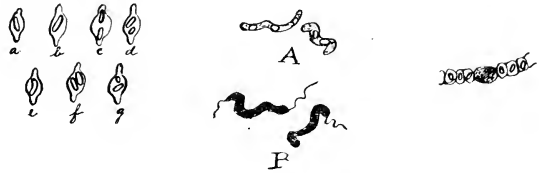


Fig. 22

Bacillus Inflatus—a, b, e, cells of clostridium form one elongated cylindrical endospore. c, d, f, g, cells with two spores of unequal size. Magnified 2,100. (After A. Koch.) Spirillum Endoparagogicum—b, vegetative cells. a, two cells, one with two and the other with three endospores. (After Sorokin.) Bacillus Tumescens—Chain of seven cells, six of which have developed one spore each, while the middle cell has remained barren. It is granular. X 1,100. (After A. Koch)

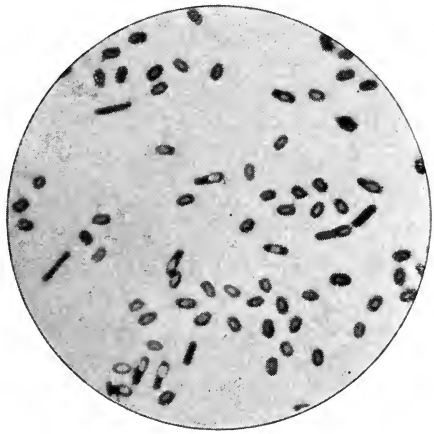


Plate 5

Photomicrograph of rods containing terminal spores, barren rods and free spores.

Spores develop in a certain position in the bacilli of the same kind, which is a guide to the student for identification. When a single spore develops in the end it is named a terminal spore, when in the middle of the cell it is called a median spore, when in a position between these two it is called an intermediate spore. During spore formation the form of the mother cell may remain unchanged, but more of swelling takes place at the point of spore formation, so that the bacillus represents a drum stick, club, or nail head, if the spore is terminal and may resemble a spindle or lemon, if the spore is median or intermediate, this form is designated as a clostridium.

The size of spores varies; they are usually oval or ellipsoidal 1μ to 3μ in length (*μ = 1/25,000 of an inch) by 5μ to 1μ in breadth, although we have reason to believe there are some very much smaller.

Fig. 23

Vibrio Rugula—Seven rods with a terminal spore. Magnified 1,020. Clostridium Butyricum—a, b, vegetative cells; d, beginning of spore formation; c, e, progress; f, h, completion; a, f, contain granular stained blue by iodine; h, sustained by iodine; g, cell with two spores. X 1020. (After Prazmowski.)

As we have stated before, the formation of spores is the means of perpetuating the species, consequently a number of bacteria are known to give rise to spores whenever the conditions are such that the bacilli cannot continue in the vegetating state. In order that the spore may be able to live through great changes in temperature, the cell wall is thick and not easily penetrated by heat or dyes, consequently in the ordinary staining methods, the spores present their oil-like refractory appearance, while the surroundings are perfectly stained.

On account of the heat-resisting power of spores, the study of this phenomenon is interesting to the canner. At one time it was believed that the bacilli as well as the spores could be destroyed by 212 degrees F., steam heat, in fifteen minutes, and no less an authority than Koch fell a victim to this theory, which E. von Esmarch overthrew. The spores of some bacteria were found to be able to sustain life after continuous boiling for six to ten hours. The same spores were destroyed, however, by a temperature of 250 degrees for fifteen minutes, steam heat directly applied. This extreme heat-resisting power led some famous bacteriologists into error. Prominent among them was Von Liebig, who built up a theory of spontaneous generation, founding it on the life which spontaneously destroyed certain infusions such as meat, milk and hay, after all life (as he thought) had been killed by boiling heat. Pasteur and Tyndall did not follow this theory, but labored to discover the cause of their failure to preserve certain infusions. After numerous experiments they were able to state positively that there were forms of life which could live through the boiling point; so the system of discontinuous heating was discovered by Tyndall and

*The Greek letter *m* is an abbreviation for the Greek word *micron*, which means small and is equivalent to $\frac{1}{25000}$ of an inch.

this method of sterilization is still used to this day as a means of sterilizing certain materials which are altered by the employment of high temperatures.

The theory was built upon the fact that the tender, vegetating forms of life were easily destroyed by temperatures as low as 160 degrees F., consequently after heating once the first day, Tyndall allowed the infusions to cool, and stand long enough for the spores to begin to develop into bacilli, when he again subjected them to a second heating, and repeated the process three times, by which he killed all, the spores having all started to germinate. While this system is useful for the sterilization of some infusions, it cannot be declared infallible, since only the aerobic bacteria (i. e., bacteria which grow in the presence of air), would develop from their spores during the intervals between the heatings. The spores of the anaerobic bacteria would not germinate unless the process were repeated in a condition where air was expelled. This would require a number of heatings, some after exposure to air for the spores of aerobic bacteria to germinate and some after the exclusion of air for the spores of the anaerobic bacteria to germinate. For ordinary purposes, however, the temperature of 250 degrees F. was found sufficient to destroy all life in material which was not altered by the heat.

Under ordinary conditions, the spores of bacteria will live for a number of years. Cold does not seem to destroy the spores and only a few antiseptics will kill them. Pressure of great power does not destroy them nor have electricity nor the X-rays proved successful destructive agents. Radium has no devitalizing power, as demonstrated by Prescott. Carbolic acid, bichloride of mercury, and hydrocyanic acid will destroy them in a few minutes, but ordinary antiseptics in such proportions as are commonly used for the preserving of certain condiments, do not destroy the spores, but produce conditions which are unfavorable for their vegetating into full-grown bacilli.

THE SPORE IS THE LIFE SEED of any given species, and if all moisture is absorbed from its surroundings, it will dry up, the membrane around it will harden, and it may cling to dust or floating particles in the air, and be wafted here and there by currents of air until it falls into a substance suitable for its germination, or it may die, although it has been known to remain alive and have power to germinate after many years of dormancy.

When it falls into a suitable medium for its germination, the spore-wall softens and a considerable amount of moisture is absorbed, which results in the development of a living cell; this will lengthen and divide, remain attached or become free, until the conditions are reached for the cells to form spores again. Spores do

not multiply, they simply furnish the life for one new cell, which will multiply.

THE GERMINATION OF SPORES is different in many cases, although it is probable that spores of a given bacillus always germinate in the same manner. The observation of spores germinating is a tedious proceeding, requiring much time and careful preparation. It is usually observed in the hanging drop culture, which is made by placing a drop of nutrient material on a cover-glass and inoculating this with a species which has formed spores in some other medium. The cover-glass is then inverted on a slide with a hollow-ground cell in the center so that the drop will hang without touching the glass, the cover-glass being sealed all around by vaseline to prevent evaporation, thus:

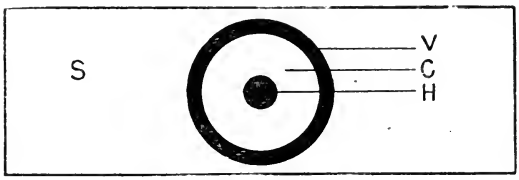


Fig. 24. Hanging Drop Culture

H—Hanging Drop.
C—Cover glass over the cell.
V—Vaseline holding cover glass over slide S.

The edge of the drop is found with the low power objective first, and then a drop of cedar oil is placed on the top of the cover-glass and the 1-12 oil immersion lens is brought down into focus. The first sign of spore germination will be a glistening of the spore, which will be seen to swell and lengthen, grow less bright, until a homogeneous cell is formed showing very little or no refraction.

The germination is accomplished in several ways.

Probably the most common method of spore germination is the growth of the bacillus from one end. The end appears to open and the young cell pushes out in the long axis of the spore. Another method is the opening of the spore at the sides, when the spore seems to split in halves, letting the young cell out in a right angle to the long axis of the spore.

Another method is the opening of the spore wall on one side through which the young cell emerges in a bent form, the middle coming out first and then the ends, causing the young cell to look like a magnet or horse-shoe.

Another method of germination is the swelling of the spore by a gradual elongation of the spore, which seems to absorb moisture, increasing in protoplasm until a fully developed cell is born, able to multiply in the regular manner.

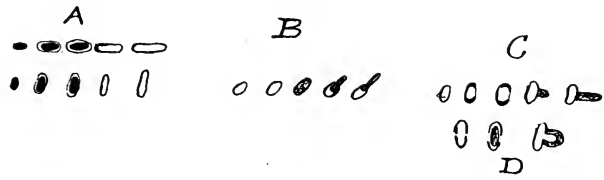


Fig. 25

A—Lengthening of the spore.
B—Bacillus growing out of the end of the spore.
C—Bacillus growing out of the side of the spore.
D—Bacillus growing out of the divided spore and in in horse shoe shape at the side.

The study of spores is most interesting to the manufacturer of food products, for the reason that they are very resistant to heat, which is usually employed to sterilize all canned goods and many other kinds of goods as well. There are some varieties of spores which live through continuous boiling for five hours and more, but are killed at 250° F. by directly applied steam heat, in about fifteen minutes. Now, this heat must come directly onto the spores, so the larger and denser the volume, the longer it takes to heat it to the center. Various liquids, such as soups, are canned, which convey the heat better than heavier materials, such as corn, peas, meats, etc., and to destroy the spores of certain classes of bacteria the temperature of 250°F. must reach the center of the package and be maintained for about fifteen minutes. For experimental tests, there are made especially for this purpose self-registering thermometers which fit into the can and remain in the center. When the temperature reaches 250°F. the mercury does not drop on cooling—owing to a constriction in the tube which holds it—until it is shaken down by gentle tapping.

Valuable knowledge may be gained by the canner from personal experiment with this device in his sterilizing processes. By noting how many minutes it requires to reach the proper temperature after the retort thermometer indicates the right temperature on the outside, he can know just how long he must process his goods to destroy the spores of such bacteria as are known to infest the particular product which he is canning. The canner must first study just what bacteria are continually present in the particular products he is manufacturing; this is found out by cultivation on nutrient media similar to those products. Later we will explain how to make these cultivations so that all kinds of germs may be isolated and identified, when grown by themselves, and experiments

made by inoculating sterile cans and subjecting the bacteria to various temperatures in order to learn their resisting power to heat, etc.

NATURE OF BACTERIA.

COMPOSITION. Water makes up a large per cent. of the composition of bacteria and is calculated to be from 60 to 85 per cent.; fats, from 1 to 40 per cent.; proteins, 10 to 15 per cent.; mineral compounds, 1 per cent.; sometimes cellulose and granulose are present, particularly in the carbohydrate species. All bacteria contain one or more soluble ferments which are compounds capable of producing fermentation, and are called enzymes. The class of bacteria which produces diseases in man and animals produces poisons called ptomaines and toxins, which may be extracted. From the non-pathogenic bacteria also the enzymes may be extracted, some of which are used in producing desired fermentations. Heat usually destroys these enzymes as well as a great many toxins from pathogenic bacteria.

The average composition of bacteria is estimated at 85 per cent. water and 15 per cent. dry matter (by Kappes). The dry matter is made up as follows:

	Per cent.
Etherial extract (fat, etc.)	4.8
Albumen	71.2
Ash	13.5
Undetermined matter	10.5

There is a very large per cent. of nitrogen in bacteria, far more than in the higher vegetable orders, and carbon enters largely into the composition of the protoplasm. The bacterial cells are devoid of chlorophyll—(the green coloring matter of the higher vegetable kingdom)—consequently, they cannot obtain the carbon, as do the higher plants, from carbonic acid gas, but are dependent upon carbon compounds. Sugar furnishes carbon, also other carbohydrates and fats, proteins, and likewise organic compounds, such as glycerin, tartaric and lactic acids.

The nitrogen of the air cannot be utilized by bacteria (one class excepted), consequently they are dependent upon nitrogenous compounds for their supply. Animal and vegetable matter is always accessible, the proteins of which furnish the nitrogen. Chemical compounds and acids can also be utilized.

The hydrogen is obtained from the same sources as the carbon and nitrogen, but the oxygen may be obtained from the atmosphere or from compounds, according to the kinds of bacteria and their environments.

Some bacteria thrive well in media which are acid; others thrive better in alkaline media.

The chemical composition of bacteria, therefore, depends to a great extent upon the nature of the substance upon which they are thriving. Certain bacteria may grow luxuriantly in an acid medium such as tomatoes and fruit juices and may remain dormant or die in an alkaline medium. Then, again, other kinds of bacteria which grow well on an alkaline medium will remain dormant or die in an acid medium. To this class the majority of bacteria belong. This difference in the nature of bacteria will throw some light on the different temperatures employed in sterilization, the character of the bacteria being determined by the degree of acidity of the material.

BACTERIA AND THEIR SUPPLY OF OXYGEN.

Many kinds of bacteria obtain the oxygen so necessary for the process of multiplication from the air, and if the air is cut off they either remain dormant or die—these are called *aerobic;* others cannot use the oxygen of the air, so they obtain their supply from organic compounds, such as the proteins and carbohydrates; these are called *anaerobic.* There are others which accommodate themselves to whatever condition in which they may be placed, if aerobic by nature they will still grow in an anaerobic state and are called *facultative anaerobes;* or if anaerobic by nature and grow in an aerobic state they are called *facultative aerobes.*

Nearly all bacteria found in improperly sterilized hermetically sealed packages are either anaerobic or facultative anaerobic. This latter class sometimes causes more violent fermentation when forced to grow in the absence of free oxygen than when growing naturally; being deprived of free oxygen the tearing down of organic compounds is accomplished with great rapidity to supply the required oxygen, while the actual multiplication is lessened. This fact is interesting to canners, as it accounts for the rapid spoilage of goods which have been improperly sterilized. Here the fermentation is much more violent and rapid than in the packages where there is a perceptible leak through which the oxygen from the air passes. It is curious that sometimes the product will be found perfectly sweet at the bottom of a can which has a large leak in the top, while the whole surface is covered with molds, yeasts and bacteria of various kinds. In this case the evolution of gas is not as great as when the can is undergoing chemical changes in the absence of atmospheric oxygen.

To the facultative anaerobes a large per cent. of losses in canned goods is due. The anaerobic bacteria, however, cause spoilage in many cases where the others are destroyed, because they belong to the soil and are spore-bearing and have the power to withstand very high temperatures and afterward develop. All anaerobes known, except possibly one species, are bacilli, that is, rod-shaped.

Bacterium Phosphorescens, Fischer

PHOTOBACTERIUM PHOSPHORESCENS.

Origin.—It is found on dead seafish, oysters, etc. Meat in butcher shops may be contaminated from these.

Form.—Short, thick bacillus, having rounded ends; almost a coccus sometimes. Usually found in pairs, but may form threads; involution forms soon develop.

Motility.—It is not motile.

Sporulation.—Has not been observed.

Anilin Dyes.—Stain readily, as does also Gram's method.

Growth.—The growth is moderately rapid. Cultures show a marked bluish-green phosphorescence in the dark.

Gelatin Plates.—The colonies are small, white and glistening, and do not liquefy; they have a sharp, irregular border; are granular, and show several concentric rings.

Stab Culture.—This shows a slight granular growth along the line of inoculation. Is most abundant on the surface, forming there a thin grayish white covering. The gelatin is colored a yellowish brown.

Streak Culture.—On agar, potato, etc., the growth is limited to the line of inoculation. The growth is very good on fish, beef, bread, fats, etc.

Oxygen Requirements.—It is a facultative anaerobe. The production of light depending upon the presence of oxygen, it is most marked on the surface growths. The intensity of the light may diminish, or may even become lost (attenuation), but may be restored by growth on suitable media, such as fish.

Temperature.—Will not grow in the incubator. May grow at 0° C.

Behavior to Gelatin.—Does not liquefy. It may ferment sugar.

Pathogenesis.—It has no effect on animals. One species is said to produce a disease in certain crustacea.

INFUENCE OF ELECTRICITY ON BACTERIA.

Cohn experimented with electricity generated by two cells, passing the current through a fermentable substance where bacteria were present and found that they were not killed, but that changes were produced which made the medium unfit for bacteriological development. Later a stronger current of electricity was tried by other investigators and some forms were killed, but the resistant forms found in milk were not affected, which dashed the hopes of those seeking a speedy method of sterilizing milk. D'Arsonval and Charrin used a current of 10,000 volts on a certain species of germ found in pus, but only a decrease in virulence was observed.

Electricity, however, is used in the manufacture of wine and cognac for maturing the flavor and not for antiseptic purposes. A certain mellow flavor is produced by pouring the liquors over plates charged with electricity.

The results of these experiments proved that electricity was not practical as a germicide, but that certain chemical reactions were produced which were inimical to bacterial growth. If salt were present in the current it would be either decomposed into acid and alkali or set free, chlorine and hypochlorites. Sometimes peroxide of hydrogen and ozone would be generated by the electric current in sufficient amounts to destroy a number of non-resistent forms. Heat also is generated which will destroy germs of the same class.

It was expected that the X-rays would prove to be of value as a germicide, but many experiments have resulted negatively. Like-

Plate 6. Bacillus Phosphorescens
Magnified 1,000 diameters.

wise it has been demonstrated that Radium does not destroy bacteria to any appreciable extent.

INFLUENCE OF TEMPERATURE.

The subject of temperature is most interesting to canners and manufacturers of food products because it opens up a way of successfully destroying the scavengers which constantly menace those products.

Bacteria grow best between the temperatures of 70°F. and 100°F., but are able to live through great changes which seem marvelous to those who have not had experience and suffered losses through their ravages.

There has been discovered one species of bacteria which multiplies at 32°F. It was discovered in 1887 by J. Forster growing on the surface of salt water fish, a phosphorescent variety. Later it was discovered by B. Fischer that the soil and also sea water contained many varieties which could grow at 32°F. A great number of these hardy varieties grow in milk and drain water.

Experimenters have tried various temperatures as low as 250° below zero, also in solidified oxygen for many hours, and some varieties lived through the tests. Severe cold is germicidal to many species.

This is interesting in connection with cold storage and accounts for some peculiar changes we have witnessed. In a general way it can be stated that the freezing point will retard decomposition of food stuffs, in fact 33° and 34°F. will keep fruits and vegetables very well for a long time, but there is loss in flavor from the formation of CO_2 (carbonic acid gas), due to the breaking up of small quantities of sugar, either by bacteria or the cell life of the fruit itself.

The temperature required to protect meats and butter is considerably lower than freezing, averaging about 13° to 10°F., but even in this temperature after a time certain changes may be noted which are not due to bacterial action, (but are due to a loss of volatile ethers which are retained by hard freezing.) Commerically, cold storage is a good method of carrying over food stuffs of many kinds, but of course decomposition will set in quickly after they are brought into warm temperatures for the reason that their exposure to germ-laden atmosphere has invited hosts of bacteria which will start their functions when brought into warmer temperatures.

It is my belief that the cold storage system will come into favor with the canner and preserver. In a series of experiments recently tried, fruits and vegetables kept nicely when frozen solid. Such products if subjected to 13°F. freeze solid and retain their flavor fairly well, but can only be used in jams and sauces, etc.,

owing to the collapse which takes place when thawed out. Even for these purposes, the system is advantageous, especially when overcrowded with raw fruits and vegetables the canner can put into cold storage his surplus, which may be made up later into marketable products, when the receipts of raw materials are less.

The cost of building a cold storage plant for every canner is too great to even be considered, but those whose factories are located within a short distance of such a plant can take advantage of it. Indeed a series of experiments along this line might prove most satisfactory.

While we have found rare species of bacteria able to live and multiply in freezing temperatures, it is interesting to note that there are varieties able to thrive at an extreme temperature in the other direction. These bacteria are non-pathogenic, that is, not disease producing, in man and animal. They are called thermophilous, or heat loving bacteria, and one species was discovered by Miquel called Bacillus Thermophilus, which multiplies at 158°F. When we remember that this temperature kills animal life and coagulates egg albumen and blood serum, we are impressed with this remarkable fact. This is an aerobic bacillus about 1μ in thickness and forms threads at about 140°F. and only thrives between the temperatures of 98° and 162°F. This bacillus is common in sewage and is found in the alimentary canal of man and animals.

There are a number of bacteria which grow well at high temperatures, most of which are found in the soil, in sewage water and in the alimentary tract. During the extremely hot weather of summer, these heat loving bacteria grow luxuriantly, and cause fermentation where least suspected.

In this connection I wish to remind our readers that they may have seen cans of improperly sterilized goods fermenting in the center of a pile where the temperature would be almost scalding. I have seen cans of corn fermenting with so much heat that I could scarcely hold a can in my hand. There has been very little investigation into this phenomenon, but some of these bacteria will be described and their resistance explained. There are certain kinds of mold which have the same characteristics as the thermophilous bacteria, and cause great loss, especially in the manufacture of catsups and sauces made from tomatoes. Some of these molds are pathogenic also, and are associated with disease in the lungs of man and in the bronchial tubes and throats of birds.

Recently we examined the sputum of a pneumonia patient and found the fungus growing; it is called Aspergillus Fumigatus and will be described later.

There have been discovered in the hot sulphur springs at Ilidze in Bosnia, two kinds of bacteria, one called Bacterium Ludwigi, which developed at a temperature above 122°F., and the

Aspergillus Fumigatus, Lichtheim

Origin.—White bread, preserves, catsup, in the lungs and air passages of birds; met with in man also.

Color.—Greenish or bluish-green growth; resembles that of penicillium very much.

Mycelium.—Mycelial threads and spores are smaller than those of A. niger.

Fruit-Organs.—The fruit hyphae are club-shaped and covered with sterigmae, from which extend rows of spores. The sterigmae are not divided. The spores are usually colorless and from 2½ μ to 3½ μ in diameter.

Growth.—Rapid. Grows best on bread.

Bread Flasks.—Low growth; at first bluish-green, but is grayish-green when old.

Temperature.—Grows best at 37-40° C. Will grow at ordinary temperature, but not below 15° C.

Pathogenesis.—Death was produced in a few days by intravenous injections of millions of spores in rabbits and dogs. Mycelia were found in the kidneys, heart and other muscles, and sometimes in the liver.

A pneumonic or pseudo-tuberculous disease is produced by the inhalation of the spores in doves and other birds. Natural affections of this kind are frequent among birds. They are met with occasionally in horses and in cattle, and sometimes in man.

In mycoses of man, the lungs, eyes, ears or nose are subject to invasion.

The Japanese utilize the growing A. Oryzae as a diastatic ferment. Rice grains are converted by it into sugar and dextrin, which when subjected to fermentation yields the national drink, Sake, containing about 14% of alcohol. Taka-diastase is a ferment which is derived from an aspergillus similar to that mentioned.

other named Bacillus Capsulatus, produced endospores which lived through a heat of 212°F. for four hours without being destroyed. We have called attention to the heat-resisting powers of spore-bearing bacteria under the head of spores. Among these are several varieties which are found on the skin of potatoes and in milk; also one variety found associated with hay, malt, etc. These hardy varieties found on potatoes are Mesentericus Vulgatus, Mesentericus fuscus and Mesentericus ruber, the spores of the last able to live through six to ten hours boiling at 212°F. Bacillus subtilis,

Plate 7. Aspergillus Fumigatus

Photomicrograph of unstained mold Aspergillus Fumigatus, which sometimes makes its appearance on food products, such as preserves, tomato sauces, etc. It is pathogenic. There are two fruit pods full of conidia near the center. Just below the larger pod is one partially disgorged. Some of the loose spores or conidia may be seen among the threads of the mycelium. Magnified 600 diameters.

a species found in boiled hay and malt infusions, gives rise to very resistent spores and gave Prof. Tyndall so much trouble in his efforts to overthrow the theory of spontaneous generation.

INFLUENCE OF LIGHT ON BACTERIA.

In a general way we may state that direct sunlight is detrimental to the growth of bacteria and is germicidal in many cases. The effect of direct rays is injurious to cultures of certain species which when thus exposed, lose their power to vegetate when returned to the dark. The effect of sunlight may be noted in many instances by its peculiar action on bacteria. Certain species which

are actively motile due to flagella (which will be described later), lose their power to move and gradually weaken and die; others which are called Chromogenic or color-bearing bacteria, (see section on pigment—producing bacteria), lose their function of producing pigments; the pathogenic bacteria lose their power to produce toxins in some cases.

It is the ultra violet, violet, and blue rays of sunlight which are so germicidal; the green, red and yellow have very little or no injurious effect upon bacteria. For extensive literature on this phenomenon the reader is referred to Diendonne (A.G.A. IX, 405 and 537).

The diffused rays of sunlight have very slight disturbing influences on bacteria; likewise arc light and incandescent influences may be observed.

The action of sunlight may produce chemical change in the medium on which the bacteria are thriving, such as the oxidation of fats, formic acid and formaldehyde and peroxide of hydrogen may be formed, which will be germicidal. In this connection let me say that these compounds are often formed in canned goods in the steam retort by oxidation, and the analyses recently made by certain state chemists were faulty in the extreme and produced the impression that these chemicals had been purposely added to the samples analyzed, when I know it to be a fact that they were not. They should have known from the very nature of the goods they were analyzing that oxidation would naturally take place at the temperatures to which the goods had been submitted, that traces of these germicides would be formed. Far be it from me to in any way discourage the efforts put forth by conscientious investigators to improve the quality of food, by condemning right methods; but when the canning industry is assailed unjustly, and with the motive possibly of gaining notoriety, it is proper to protect the manufacturers and furnish them with information to refute false analytical reports. I personally prepared two of the samples, absolutely pure in every respect, which were submitted to the state chemists and their report showed that formaldehyde and benzoates were present, which was false in the sense that they had been added, and that report gave a wrong impression to the public. They should have known that these had not been purposely added, but that the faint traces were due entirely to oxidation produced by steam heat 250°F., and that the product would naturally yield traces of such preservatives.

To return to our subject, we have found that sunlight is germicidal and that diffused light and electric light are slightly injurious to bacteria. This will overthrow the idea that wrapping with brown paper and storing in dark places fruits and vegetables canned in tin and glass would prevent fermentation. Bacteria grow

best in the dark and the only value in the ancient custom, was protection from dust and protection of color, which sunlight injures to some extent.

MOTILITY.

My first impression when observing the rapid, almost marvelous movement of certain bacteria was, that they must surely belong to the animalcules. So rapid was their movement that the eye could not possibly follow them. Having focused the edge of a hanging drop culture of the typhoid bacillus, I tried over and over to move the slide and keep in focus with the fine adjustment of the microscope, the swift moving germs, but they quickly passed out of the field or dropped so deep in the fluid that it was impossible for me to follow them. Other active varieties also are apt to create the impression that they are not a part of the vegetable kingdom, but their life history and manner of vegetation dispels the doubt.

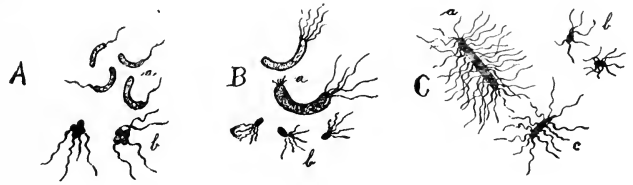


Fig. 26

A—Monotricha
a—Cholera Bacilli.
b—Sarcina Pulmonum.

B—Lophotricha.
a—Spirillum Undula.
b—Back Syncyaneum.

C—Peritricha.
a—B. Vulgatus.
b—B. Prodigiosus.
c—Typhoid Bacilli.

Pasteur regarded the "vibrion butyrique" as an animalcule in his early researches, but the higher order of algae have motile spores and their movements are extremely rapid too, showing that the vegetable kingdom does have actively motile species, and motility could not be an argument in opposition to their place in this kingdom.

There are two kinds of motion observed in bacteria, one a molecular motion, sometimes called "Brownian motion;" the other is called independent motility. The former is purely a physical motion and may be noticed in many particles held in liquid suspension. There seems to be an oscillating motion peculiar to many micrococci and bacilli, a rotary or orbit motion, due perhaps to the vibration of the fluid. The molecules of the fluid may be round like shot and roll over and over as slight chemical changes occur or by shock or by the influence of the earth, etc. Certainly no one has ever seen a molecule of water, but it seems probable that the two atoms of hydrogen and the one atom of oxygen might combine into a spherical molecule, and if such be the case there would prob-

ably be great oscillation going on continually, thus accounting for the peculiar movement described as "Brownian movement." Cohen made some experiments which seem to lend color to the theory given above. Gelatin was gradually added so that all the particles held in suspension became quiet and he noticed that all bacteria which had no independent motion, also became quiet, while the bacteria which had an independent motion moved quite freely.

The subject has not been fully investigated and the theory of molecular oscillation will probably stand.

Bacteria which have independent motion are endowed with organs of locomotion which are most interesting to study. These organs are called by different authors *flagella, celia,* or *whips,* which grow out either from the ends or sides of the bacilli or both. The number and kind of flagella often determine the species to which a certain bacillus belongs. These organs of locomotion were first discovered by Ehrenberg in 1836, and more carefully studied by Cohen. It was supposed for a long time that only bacilli were thus endowed, but in 1887 and 1890 Loeffler and several others claimed to have discovered certain cocci which were motile, but this is doubtful.

These flagella are not visible when the bacteria are stained with ordinary dyes; the most powerful objectives do not clearly show their presence unless special staining is done to bring them into view. Some bacteria have only one polar flagellum. Others have two or may have a bunch at the end; still others have them evenly distributed over the entire surface of the cell, sometimes as many as a hundred. The number and size of the flagella depend upon the age of the cells. Usually they are best seen and studied in cultures 24 to 36 hours old.

Flagella are very thin, hair-like appendages, so fine that the dyes must be piled upon them to bring them into vision; they vary in length from two to twenty times the length of the bacillus and seem to derive active power from the cell itself so that they impart peculiar motion to the rods; some having a wabbling motion, others a creeping motion, others a snake-like motion and still others turn somersaults and whirl with a rapidity truly remarkable; and the spirochetae have a boring or corkscrew movement. Bacilli are classified with reference to the number of flagella (see Fig. 26) into four groups—*monotricha,* having one flagellum at the end; *amphitricha,* having a flagellum at both ends; *lophotricha,* having a bunch of two or more at the end; and *peritricha,* having the whole surface arrayed with the hair-like whips.

These propelling organs are so delicate that they are easily injured and fall off or become looped when disturbed by external influences. When they are thus injured they disappear very soon and are apparently dissolved in the fluid surrounding them. They

often fall off just at the time of spore formation. This is true of nearly all species excepting some anaerobic bacteria. Old cultures therefore are not suitable for the demonstration of flagella. I want to emphasize this point because the beginner will have considerable trouble, even under the most favorable circumstances, to stain the delicate celia property; a young culture is always to be preferred.

The influence of chemicals, antiseptics, salicylic acid, benzoates, etc., is such as to cause loss of motion or death to bacteria, but loss of motion does not always mean that the bacteria are dead. Often they may be transplanted into favorable nutrient media and become as actively motile as before. The enzymes and toxins which are the products elaborated by the bacteria themselves when growing in a favorable substratum (nutrient substance), often cause loss of motion and the *agglutination* (gathering in bunches), tests are made possible by this characteristic. This phenomenon is most valuable to the bacteriologist in determining cases of typhoid fever. Where the patient has typhoid fever the poison very early is distributed through the blood and this poisoned blood will cause the agglutination of the typhoid bacilli when they are introduced into a drop of the serum, diluted with bouillon.

Sometimes the microscopist meets with peculiar bodies in a field or view where a pure culture of motile bacteria is being

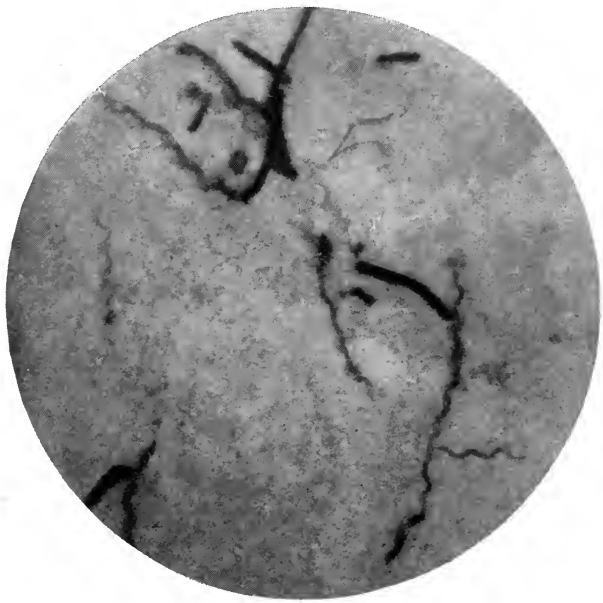


Plate 8. Giant Whips of Malignant Oedema

Photomicrograph showing rods and giant whips. These large twisted bodies are visible in the water of condensation without staining. Nearly all motile anaerobic bacteria produce these. Just what they are has not been determined, but their presence is interesting. Magnified 2,000 diameters.

studied. Loeffler in 1890 observed large spindle-shaped bodies resembling twisted hair. Later in 1893 Novy observed these same bodies, which he calls *"giant whips,"* while studying various anaerobic bacteria. Fischer, Sakharoff and Sames also describe these large spindle-shaped bodies.

The spirals are very large, varying from 20 to 100μ in length, and may be observed without resorting to stains, in the water of condensation of freshly inclined agar in tubes inoculated with cultures of motile bacteria. They are motionless and have a wavy appearance, or resemble a spindle wrapped in twine.

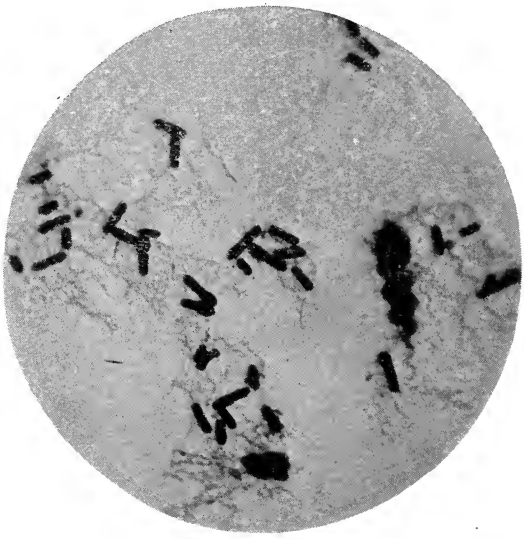


Plate 9. Giant Whips of Bacillus Butyricus Frumenti

Photomicrograph of Bacillus Butyricus Frumenti, showing ordinary flagella and also a bunch of giant whips greasly resembling a bunch of hair. This is an obligative anacrobic bacillus found in corn and was abtained from a swelled can of corn. The pressure of gas created by this organism is enormous, sufficient to burst the cans. Stained by our special method from a young growth on 2 per cent. glucose agar. Photographed through a 2 mm. oil immersion objective using acetylene radiant. Magnified 1,200 diameters.

The staining and demonstration of flagella will be fully explained later. It is accomplished only with great care and fine mountings are obtained only by practice and patience.

CHROMOGENIC BACTERIA.

Chromogenic bacteria are the species which produce pigments or colors of various shades and play an important part in the deterioration of food products. There are two classes—Chromoparous and Cromophorous.

CHROMOPAROUS, or color producing bacteria, are themselves colorless, but they produce pigments of various shades which give distinct colors to the food stuffs on which they are growing.

THE CHROMOPHOROUS group produce colors within the cells, and may or may not give off the color to the media upon which they are growing. The colors produced by the chromogenic bacteria can be distinguished by their behavior towards solvents. The same bacteria always produce the same color when grown in the same temperature and in the same media. Identical colors may be produced by more than one variety, but the varieties may be differentiated by the chemical reactions of their pigments.

The red colors are frequently seen like drops of blood on many cooked vegetables, such as potatoes, starch, flour, egg albumen, carrots, meats, milk, onions and others which enter into the formulas of soups, sauces, etc. Cane sugar syrup is often affected by these colors. The oldest known bacterium producing a red color is Bacterium prodigiosum. Cultures of this organism were used by magicians years ago to imitate blood spots on bread, whence its name of "Bleeding bread" originated. There are several forms closely allied to this, which produce various shades of red from a pink to a deep brown red.

Milk is particularly subject to changes in color by these organisms; also cheese; but such colors in these products may be produced by other causes such as blood, or madder (Rubia tinctorum in the fodder fed to the cow. Sometimes cheese assumes a red color from a purely chemical change caused by the oxidation of iron compounds which develop during the ripening of the curd. These chemical changes are quite easily distinguished from the red colors produced by bacteria. More frequently cheese owes its red discoloration to mold fungi belonging to the group of Eumycetes.

Dried codfish is very susceptible to red colors so that it resembles salmon. Three different varieties which cause the trouble have been studied by Le Dantec, who states that the loss is estimated to be about ten million francs annually, since the people believe that codfish affected with this color is poisonous.

YELLOW COLORS are produced by a number of bacteria, but very few foods are affected by them excepting milk. Milk sometimes develops a pale orange yellow and the bacteria causing it were first studied by C. J. Fuchs in 1841 and by J. Schroeter in 1870, the latter isolating two microbes named Vibrio Xanthogenus and Bacterium synxanthum as the cause, but he claimed that they were found in milk only after boiling. The yellow pigment produced by these germs is soluble in water, but not in alcohol nor ether.

BLUE COLORS are produced by several varieties of microbes and the principal foodstuffs affected are milk and cheese. Milk and cheese enter into the formulas for manufacturing so many different varieties of food products that the manufacturer of soups

Bacillus Prodigiosus

MONAS PRODIGIOSA, OF EHRENBERG. MICROCOCCUS PRODIGIOSUS.

Origin.—It is found on starchy substances, such as rice, potatoes, moist bread; also on meat, albumen, milk, etc. Causes at times local epidemics, infecting foods, as bread, sausages, meat, etc., to which it gives a pinkish or red color. Bread so affected has been called "Bleeding bread."

Form.—A short rod, slightly longer than it is wide; it sometimes forms threads, especially in slightly acid media or in old cultures; usually single or in pairs.

Motility.—It shows no motion ordinarily except a marked Brownian movement. The slimy character of the growth decreases in acid or very dilute media, and a slight motion may be observed. It has numerous long wavy flagella.

Sporulation.—This has not been observed. It shows marked resistance to dessication.

Anilin Dyes.—Stain readily.

Growth.—The growth is very rapid.

Gelatin Plates.—Deep colonies are round or oval, light brown in color and with sharp border. The surface colonies are irregular, with rough border, granular, have reddish center, and are surrounded by clear, liquefied gelatin.

Stab Culture.—The liquefaction is rapid and funnel-shaped, and extends along the whole line of inoculation. A red scum is formed on the surface of the liquid, which on settling colors the entire contents a bright red.

Streak Culture.—On agar, it forms a spreading growth which is moist and abundant and of an intense red color, which is non-diffusible. On potato, it grows very rapidly, producing slime and a pigment, which, when old, has a metallic, fuchsine-like luster. Odor of trimethylamin. On blood-serum, growth is same as on agar, with liquefaction.

Milk.—Growth takes place and the fat globules hold the pigment in solution. Coagulation results.

Oxygen Requirement.—It is a facultative anaerobe.

Temperature.—It grows best at ordinary room temperature. It ceases to form pigment in the incubator and may lose this property temporarily, i. e., become attenuated.

Behavior to Gelatin.—Rapidly liquefies as the result of formation of a soluble peptonizing ferment. In acid media this liquefying property may be diminished or temporarily lost.

Aerogenesis.—It has a strong odor of trimethylamin on potatoes, and ferments sugar solutions.

Pigment Production.—A bright red pigment is formed on various media, and this is soluble in ether, alcohol, chloroform, etc. This pigment is formed only in the presence of air and at ordinary temperatures—not at 37°.

Pathogenesis.—It is non-pathogenic. In large amounts its soluble products may have a toxic action. The cellular proteins may induce suppuration. Animals which are not susceptible to malignant oedema may be rendered susceptible by an injection of this bacillus. An injection of this bacillus saves rabbits inoculated with anthrax.

and sauces will no doubt be interested in these phenomena, which he has perhaps frequently met with.

The principal microbe which produces blue colors in milk is named *Bacillus Cyanogenus;* but blue discoloration may be observed also in freshly drawn milk where the cow has fed on the flowering rush (Butomus umbellatus) which contains a blue coloring matter frequently carried into the milk from the stomach of

Plate 10. Bacillus Prodigiosus, Flagellated
Magnified 1,200 diameters.

the cow through the arteries and mammillary glands. The distinction can easily be made between these two causes by adding a small quantity of each kind of milk to normal milk. The one containing the Bacillus Cyanogenus will soon produce the blue color in the normal milk, while there will probably be no change noticeable in the other.

Bacillus is a motile organism requiring oxygen for luxuriant growth; the rods measuring from .3μ to .5μ broad to 1.4μ long. When a dairy becomes affected with this organism it is most difficult to get rid of it, often requiring complete changes in all utensils and thorough disinfection. This microbe grows well on vegetables which have been cooked, such as rice and potatoes, which are used in various formulas by the canner.

Bacillus Cyanogenus, Fuchs (1841)

BACILLUS OF BLUE MILK.

Origin.—Found in blue milk.

Form.—Small, rather narrow rods, having slightly rounded ends, two to three times as long as wide. Frequently found in paris; rarely in threads.

Motility.—It is very actively motile; has bunch of whips at one end.

Sporulation.—The small terminal bodies resembling spores are most probably involution forms. True spores may form on althea or quince jelly.

Anilin Dyes.—Stain readily.

Growth.—Rapid.

Gelatin Plates.—The deep colonies are round, having sharp, smooth border; contents are yellowish and granular. The surface colonies are round, moist, elevated, convex masses, finely granular and dark in color; at times they may be thin and spreading, having an irregular border.

Stab Culture.—In the lower part of the puncture there is little or no growth. There is a thick, moist, dark-gray, spreading growth on the surface. Gelatin is colored a dark steel-blue, the shade varying with the reaction of the medium, being quite blue in neutral or acid media and dark, or even black, in very alkaline media. It becomes dark colored when old.

Streak Culture.—On agar, a dirty gray, thick, moist covering is formed, the medium becoming dark colored. On potato, a similar growth is formed which rapidly spreads and becomes colored. On blood-serum, it forms no color.

Milk.—It produces no acid or coagulation in sterilized milk, but the milk is colored a slate-gray, which turns blue with acids. In unsterilized milk, in the presence of lactic acid bacteria, the color is sky-blue. This color develops from casein and not from lactose. In bouillon or milk containing 2% of glucose, lactic acid and a fine blue color are formed. Lactose is not converted into an acid.

Oxygen Requirements.—Aerobic.

Temperature.—Grows best at ordinary temperature, but will grow in incubator. The pigment is most marked when it is grown at low temperatures, 15° to 18° C.

Behavior to Gelatin.—Does not liquefy.

Pathogenesis.—No effect on animals.

GREEN COLORING matter is excreted by quite a number of bacteria, some of which in the presence of phosphates give the green color while in other media their natural blue color is predominant; among these may be mentioned Bacillus pyocyaneus, which causes the green color observed in meat which has been exposed to the air. This is the same organism which gives rise to the green pus often seen on wound bandages. Bacillus butyri fluorsecens, a fission fungus, was discovered by Dr. T. Lafar in 1891 as the cause of green discoloration sometimes seen in butter. Among the more common varieties which produce green pigment, the following may be mentioned:

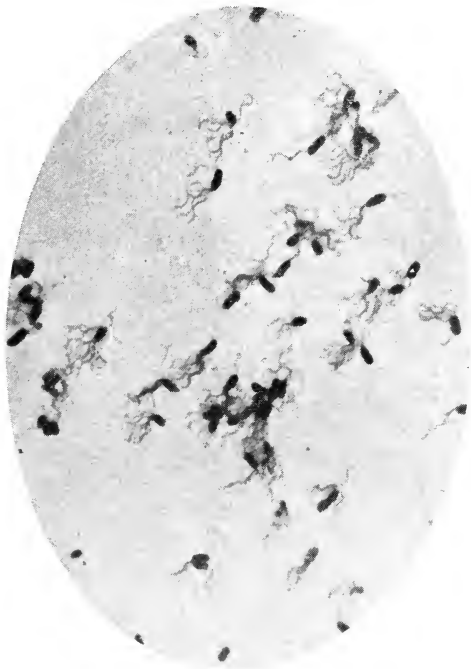


Plate 11. Bacillus Cyanogenus, Flagellated

Magnified 1,000 diameters.

Bacillus fluorescenes putidus, Bacillus fl. tenuis, Bacillus albus, Bacillus viridans and Bacterium syncyaneum; while various molds present a green appearance but do not impart their coloring to the substratum upon which they grow. The mold Penicillium glaucum gives the green color to Roquefort, Gorgonzola, Stilton and Brie cheese. There have been discovered some twenty-seven species of Penicillium. The green observed in several kinds of cheese it not due to bacteria nor fungi, but to copper which was absorbed from

Penicillium Glaucum

Origin.—It is widely distributed in air, water and soil. It is said that sixty per cent of the mold contaminations in the laboratory are due to it.

Color.—Whitish at first, changing to a bluish-green later.

Mycelium.—Is composed of straight or slightly wavy mycelial threads horizontally arranged; from these the fruit hyphae rise vertically.

Fruit-organs.—The ends of the septate fruit hyphae are forked; they are covered with sterigmae, sometimes called besidia. Each of these sterigmae bears a row of eight spores or conidia, giving to the whole the appearance of a brush. The spores are about 3.5 μ in width.

Growth.—Rapid.

Gelatin Plates.—Whitish floccules are formed by the colonies; these gradually increase in size; at the same time the center becomes a green color. The gelatin is liquefied early. The above characteristics may be seen by means of a low objective.

Bread Flasks.—A low, finely flocculent covering is formed, white at first, but changing to green later.

Temperature.—Optimum, from 22° to 26° C. will not grow at body temperature.

Behavior to Gelatin.—Liquefies slowly.

Pathogenesis.—No effect on animals. It often develops on grapes and causes a marked alteration in wine. Gives rise to diastatic and inverting ferments. It is said to be used in the preparation of Roquefort cheese.

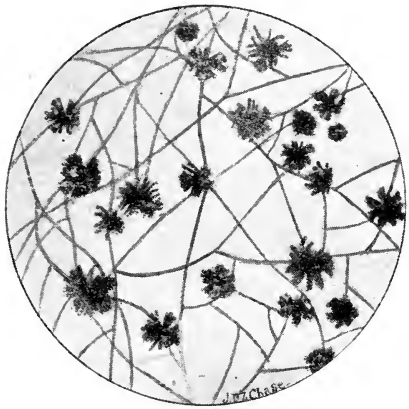


Plate 12. Penicillium Glaucum

Magnified 600 diameters.

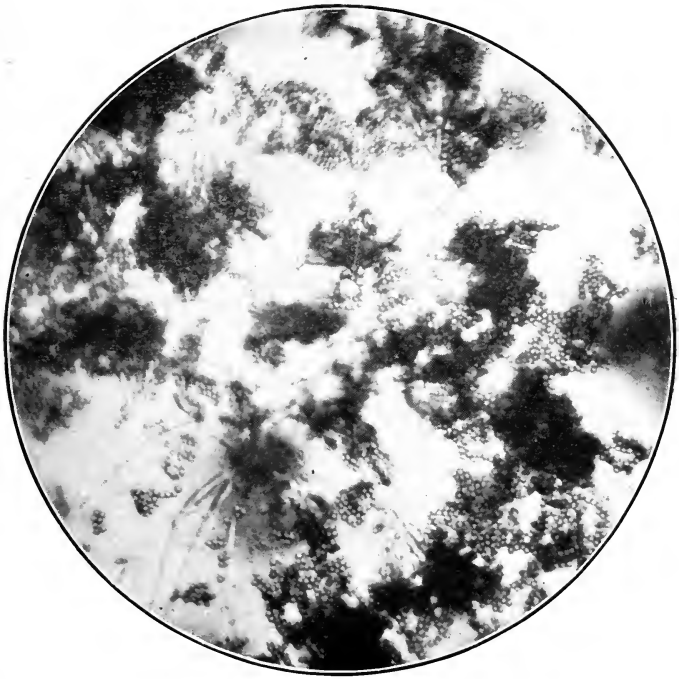


Plate 13. Penicillium Glaucum

Photomicrograph of a bluish green mold belonging to a species of Penicillium, which was found on the surface of jelly. The photograph was made of the living plant and shows the mycelium, hyphae and spores. The spores are flat on both sides and are capable of setting up fermentation when submerged in fruit juices. It will form alcohol, some acid and phenol-like bodies. Magnified 500 diameters.

copper utensils, in which the milk had been kept. The lactic acid formed, attacked the copper, which turned green in the yellow cheese.

Canners of meats should guard against the use of meat which shows any green discoloration. The presence of the green pigment indicates that the meat has been exposed to warm temperature and may have more deadly parasites flourishing on the surface and in the tissues than the color-bearing germs. There are various bacteria and fungi which produce other colors such as black smut and white spots. Packers of corn often experience trouble with black

Plate 14. Yellow Mold

Photomicrograph of a beautiful yellow colored mold isolated from the surface of California tumbler jelly. This is a very rare species and grows in almost similar manner as Penicillium, having the branched hyphae and the long rows of conidia or spores at the end. This specimen was photographed from the edge of an agar growth in the living state, sunlight being used in two ways, both direct and by transmission, giving the plate a beautiful relief-like effect. Magnified 500 diameters.

spots throughout their cans, sometimes due to these, which will be fully described under the head of Corn Packing. The packers of canned lobsters have had considerable trouble at times with black discoloration, as also do packers of various sea foods, all of which will be described in their proper places.

There are varieties of bacteria which produce violet and purple colors which are useful in the manufacture of indigo and other shades, but have no importance in the manufacture of food products.

BACTERIA PRODUCING SLIME AND ROPINESS IN FOOD PRODUCTS.

Viscous fermentation is a most important study for the canners of molasses, syrups and vegetables, such as peas, string beans, asparagus, etc. Grape sugar is often split up by Invertin, produced by several varieties of bacteria which form mucinous capsules and grow in zoogloea or masses all united. There are cocci which grow on some foodstuffs without forming the gelatinous capsules. The capsules are formed as a sticky envelope around the cocci, and sugar, lactose, maltose and dextrin seem to favor their development. The capsules are stained by special dyes and are not revealed by ordinary staining.

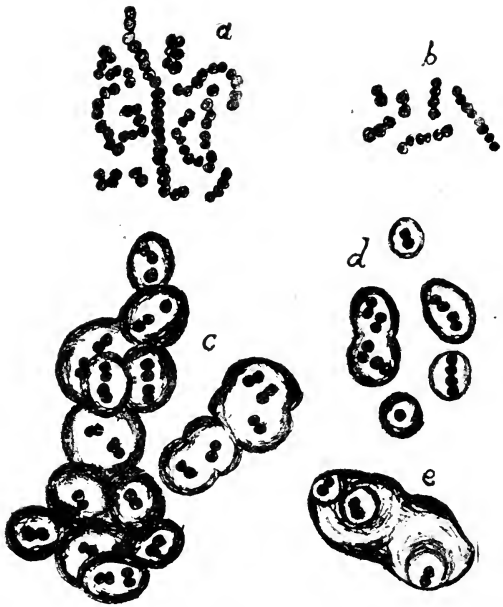


Fig. 27. Leuconostoc Mesenteroides

a. b.—Chains of non-capsuled variety. c. e.—Cells with gelatinous capsules in various stages of development. (X 1,200. After Liesenburg and Zopf.)

The principal cause of this viscous fermentation in cane sugar are the Leuconostoc mesenteroides, which were studied by Van Teighem in 1878, who published his researches, but he probably had several varieties mixed, as his drawings show chains of cocci and diplococci. In 1891, however, C. Liesenberg and W. Zopf obtained pure cultures of the organism and found it to be a coccus 0.8μ to 1.0μ in diameter.

This germ does not develop the mucinous matter when growing on nutrient substances free from sugar, but when grown on

vegetables like peas, beans, beets and carrots, which contain saccharose and dextrose, zooglea forms appear, somewhat dry at first, afterwards becoming softer and sticky. Frequently whole vats of molasses will be contaminated by this microbe and when it acts on cane sugar syrup, it produces the invertin which retards crystallization. The capsule which surround it protects it and enables it to withstand high temperatures.

Another cause of viscous fermentation is the Bacillus Viscosus Sacchari, which differs from the last organism described in that it converts the media on which it develops into a viscid mass and does not form the envelope around the cell.

There are several other varieties which cause viscous fermentation, viz.: Bacillus megatherium, Bacillus fluorescens liquefaciens, Bacillus vulgatus and others which produce changes in saccharine products, giving rise to mucus, amyl alcohol and invertin. Milk frequently becomes ropy or slimy so that it can be lifted up in long stringy threads, sometimes a yard in length. Alcohol and acetic acid are often generated in this kind of milk by two organisms isolated by E. Duclax, called by the generic name Actinobacter or lustrous bacteria.

Bacillus mesentericus vulgatus (Flugge) and Bacillus pituitosi (Loeffler) are most frequently the cause of ropiness, but recently I have observed a short, plump capsuled bacillus in cream which had become ropy. This bacillus when grown on nutrient agar develops the capsules and takes the stains quite readily. It resembles the Bacillus lactis viscosus, discovered by L. Adametz in 1890. All these organisms develop at times in milk which is allowed to stand in a warm temperature; this is easily avoided, but there is another organism which develops ropiness at a lower temperature. It is a large micrococcus about 2μ in diameter, easily killed by boiling temperature.

The peculiar flavor of Edam cheese is due to a fission fungus called Streptococcus hollandicus, cultivated in pure cultures by the Dutch dairymen and cheesemakers. Milk, when sown with this fungus, soon becomes ropy and the ropy whey is made into the famous cheese.

In Finland, Sweden and Norway the milk pails are rubbed on the inside with the leaves of the butterwort (Pinguicula vulgaris) and the cows are fed with the plant. The leaves of this plant are infested with the micro organism used by the Dutch cheesemakers, consequently the milk is soon ropy, and this thick milk is a commercial article among the Scandinavians.

Soapy or frothy milk with a slimy sediment is due to a microbe called by Weigmann, Bacillus lactis saponacei, usually the result of unclean bedding for cows. Many of the impurites in milk are due to the uncleanly methods of dairymen, and it is hoped

Bacillus Mesentericus Vulgatus, Flugge

POTATO BACILLUS.

Origin.—They are widely distributed in the soil, on the surface of potatoes, in faeces, putrid fluids, milk, water, etc.

Form.—Small, thick rods, having rounded ends. Usually found in pairs; may form threads.

Motility.—It is actively motile, having numerous flagella.

Sporulation.—Large, medium, roundish spores are readily formed. Globing describes one variety which showed enormous powers of resistance, withstanding the action of steam heat for five to six hours.

Anilin Dyes.—React easily, as does also Gram's method.

Growth.—Very rapid, resembling in many respects that of the hay bacillus.

Gelatin Plates.—The colonies are yellowish-white, slightly granular, with irregular borders, liquefying rapidly and extensively.

Stab Culture.—Growth along entire line of inoculation, liquefaction being more energetic in the upper part. The liquefied gelatin remains turbid for some time. A thin, grayish, folded scum is formed on the top.

Streak Culture.—On agar a dull white or grayish growth is formed. The most characteristic growth develops on potato. The surface is rapidly covered with a thick, white, strongly folded, coherent growth, which later becomes a dirty brown or red color.

Mild.—Casein is coagulated and peptonized, and starch is inverted.

Oxygen Requirements.—Aerobic.

Temperature.—Growth at ordinary or at higher temperatures.

Behavior to Gelatin.—Liquefies rapidly.

Pathogenesis.—No effect has been observed.

There are several varieties of potato bacilli, some forming a red and others a brown growth on potato. The spores of the potato and hay bacilli are extremely resistant—it may require an exposure of ten hours or more to steam to insure sterilization when the material is in a small mass, not in a fine state of suspension.

that more stringent laws may be passed to keep this universal food product purer and more wholesome. Milk is so extensively used in the manufacture of delicate soups, sauces and table delicacies that its special study is desirable.

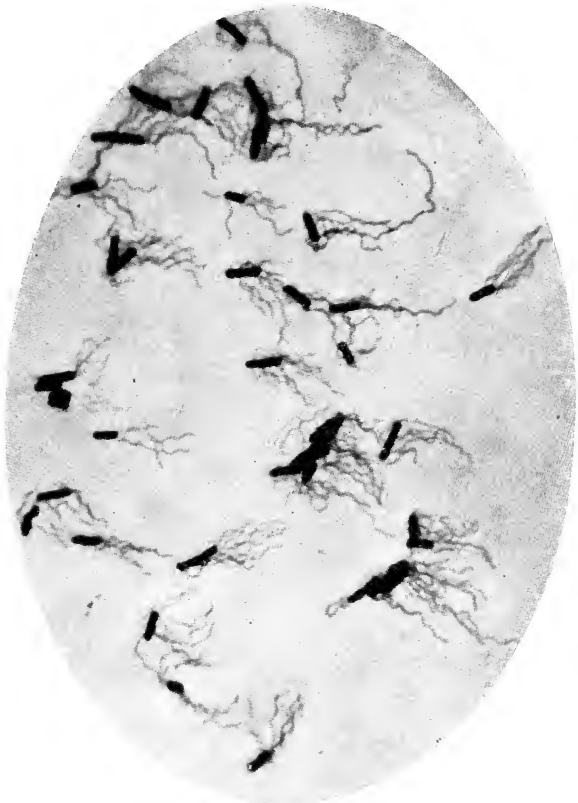


Plate 15. Bacillus Mesentericus Vulgatus, Flagellated

Magnified 1,200 diameters.

In the manufacture of Worcestershire sauce, wine is used frequently, and is sometimes found to be ropy and slimy, the flavor greatly injured. The study of this subject is one more for the wine maker than the manufacturer of food products, yet it is well to know that such wine is not fit for use and the trouble is due to fission fungi studied by Pasteur, and are Bacillus viscosus vini and other similar species.

The same scientist investigated the cause of beer and wort becoming ropy and found a fission fungus which he named Micrococcus viscosus. This organism does not give rise to violent fermentation, but seems to perform its functions in the development of a

gelatinous viscid envelope which spreads through the whole liquid. H. Van Lear obtained pure cultures of two varieties, naming them Bacillus Viscosus I and II, differing in the quantity of carbon dioxid liberated and the amount of viscous matter; the first produces yellow patches and the second a brown formation.

These two organisms, contrary to the general character of viscous ferments, do not require sugar in very large quantities to produce the gelatinous envelope, in fact sugar is injurious to them. In the manufacture of malt and cider vinegar these organisms make their appearance, frequently causing a great deal of trouble. The alcohol is favorable to their development but the acidity is germicidal. About 2 per cent. acid stops development.

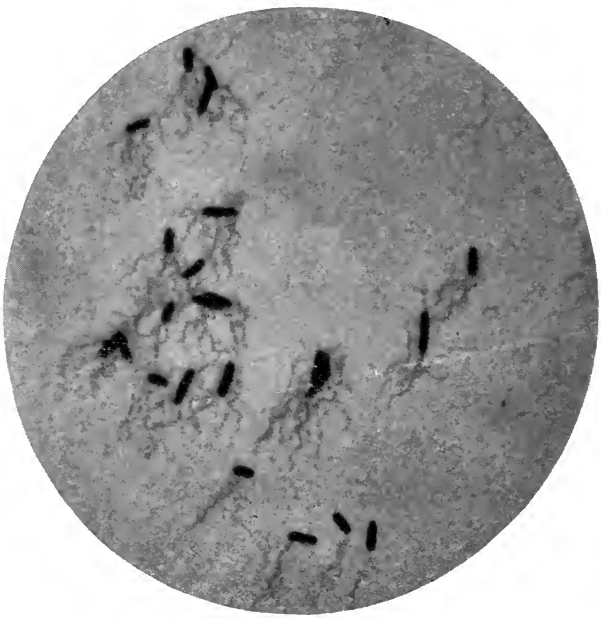


Plate 16. Bacillus Vulgatus Viscosus, Flagellated

Photomicrograph of a slime-forming, actively motile bacillus. This is a spore-bearing organism similar in some respects to Bacillus Vulgatus in its formation of slime. Incompletely sterilized molasses is fermented and rendered very viscous by the decomposition of sugar. The flagella are stained by precipitating the slime with chloroform and staining as usual, although many difficulties beset the microscopist. Magnified 1,000 diameteas.

Viscous fermentation is not confined to the family of bacteria (Schizomycetes) alone, but may be caused by some of the Eumycetes or yeast and mold fungi as well.

The canner and preserver has difficulties of this nature, particularly canners of molasses, syrups, peas, beans, corn, asparagus, etc. Owing to the heat resisting power of many species which are protected by their gelatinous capsules, sterilization is sometimes difficult. To be sure the temperature may be increased and the

time lengthened, but the quality is thereby injured. It would be no trouble to increase the process of peas to one hour at 250°F., which would perfectly sterilize them, but the result would be more of a soup than canned peas—they would all cook to pieces. The remedy lies in another direction; the raw product must be properly cared for, and not allowed to stand exposed to these scavengers. There has been considerable carelessness in this matter in the past. Raw material often stood exposed until the slimy formation could be detected by the hand. I remember when the new pea vining machinery first came into use, the hulled peas were hauled several miles on wagons in baskets eight inches deep. They were unloaded and piled up for several hours before they were canned, and the baskets were very slimy and the peas stuck together so that the grading machines were scarcely able to properly sort them into various sizes. The cans opened after the season were ropy and the peas were flat and had lost their delicate flavor.

The canners of molasses who do not take proper care of the unrefined molasses will experience great difficulty in preventing fermentation. They have been resorting to preservatives as a means of preventing fermentation and overcoming the difficulties with which they are beset through careless methods.

CHAPTER III.

Principles of Bacteriological Technique

Methods of Cultivating Bacteria. Artificial Media. Method of Cultivating Anaerobes. Methods of Simple Staining. Method of Staining Flagella. Method of Making Photomicrographs.

When foodstuff is attacked by bacteria there may be and generally are, several varieties involved, and it is essential that these varieties be separated and studied in pure cultures under all conditions. If the canner is having losses from swells and sour goods, he is anxious to know what is the cause; the preserver has mold appear on the surface of his jellies and preserves, and he is mystified as to the cause; the packers of corn and such special foods as lobster, and fishballs, find the contents spotted black and the juices are turned dark, and the mystery deepens; the packers of meats and fancy soups sometimes receive notice of ptomaine poisoning, where certain cans of their goods are held responsible, and they are totally in the dark as to the cause; bottlers of tomato catsup suddenly lose a large per cent. of their goods, where every detail of the work seems to have been carried on as in former years; picklers are surprised to find soft pickles, where their old and tried methods of salting have been followed closely. In fact there is hardly a single line of goods but that shows signs of spoilage at certain times under the most peculiar circumstances. Now when this spoilage occurs there is something to blame, and if you have carefully watched your pack, you will have received warning of brewing trouble. A can will swell and you pick it up, turn it over and look for a possible leak; the capping and tipping are smooth; the top and bottom appear all right, but the seam looks as though there might be a leak, and you pass over the matter; this may be a warning that the process is wrong, and if you let the matter drop you may find out suddenly that a large per cent. of your goods are going wrong. When a can shows signs of swelling a thorough bacteriological examination ought to be made at once. If the can is only a leak, there will be found growing inside various germs, which would have been destroyed in a single boiling process, and this would be fairly conclusive evidence that there was a leak somewhere in the can, possibly so small as to escape the most rigid scrutiny. If the can is a swell caused by an underprocess, the plate culture method will show only spore-bearing species of bac-

teria, and if this be the case, the process must be examined carefully; first, to see if everything is in good working order; second, a careful test of thermometers and gauges; and third, to see if the processor is giving the goods the required time and temperature. If all these are found to be correct, then cans must be inoculated with pure cultures of the bacteria and then incubated at 98°F. If they swell they show insufficient sterilization. Sometimes the cans do not swell, but still contain germs, which will decompose the sugar into acid, which phenomenon is known among canners as "sour goods," so it is well to cut open a few cans and streak some Petri dishes containing nutrient agar and gelatin, and put part of these in the anaerobic culture apparatus, and others are to be grown in the incubator, covered, but allowing circulation of atmosphere. Hanging drops of the liquid in the cans should be made and carefully watched under a 1-12 oil immersion lens. If living bacteria are numerous, they may be seen to move rapidly through the fluid.

Nearly all the spore bearing bacteria are motile, possessing, usually, numerous flagella, which we have reproduced in various photomicrographs throughout this work. As we have previously stated, the flagella are not visible when examining living bacteria; they are seen only after staining according to special methods which we will describe later in this chapter.

The preparation of nutrient media for cultivating bacteria is essential for bacteriological research and for general purposes the following formulae are used, but for special study the best nutrient materials are made with the fluids of such goods as are spoiling; for instance, the bacteria found in spoiled peas should be grown upon the sterilized juice of peas, or if it be spoiled corn, the medium should contain as a base the filtered sweet corn juice, etc.

ORDINARY MEDIA.

BOUILLON. This is made from fresh lean meat juice and is used generally to demonstrate certain peculiarities of various species, to differentiate species, and aid in their identification. Sometimes the bouillon is densely clouded, sometimes only slightly; a thick sediment may form, and this precipitate may be easily diffused by shaking, or may be too heavy; some cultures form pellicles or skins over the surface, and these may be easily broken (by shaking the tube) or may be very tenacious. The indol reaction with sulphuric acid is best demonstrated in a bouillon culture. Bouillon is an excellent medium for growing the various germs which produce ptomaines and toxins; the germs may be filtered out by forcing the liquid through a porcelain, or Chamberland filter, and the analysis may be made of the filtrate for such poisons. Bouillon cultures

are made of the various germs which are used in agglutination tests, described under Typhoid, in Chapter V.

FORMULA. 500 grammes (about 17 ounces) of lean beef are cut up into small pieces; there must be no free fat; the meat is covered with 1,000 cubic centimeters, or one litre (about one quart), of distilled water, and let stand in refrigerator 24 hours. Squeeze out all the juice possible from the meat and strain through flannel, or, better still, gently simmer over flame for one hour, and then strain. The flame should not come directly in contact with the bottom of the enameled pan, so a sheet of asbestos is placed between the flame and the pan or one with a double bottom may be used. If, after this, the volume is short of 1,000 c.c., add enough distilled water to make up the amount, then add 10 grammes of Merck's meat peptone and 5 grammes of common salt. Put the mixture into the autoclav or process retort and raise the temperature very slowly up to 240°F. and hold for 30 minutes; after removing, strain through four thicknesses of flannel and let stand until cold, then filter through ordinary filtering paper. Test the bouillon with blue litmus paper and it changes the color to red, which shows an acid reaction. As most bacteria grow better on slightly alkaline media, prepare a strong solution of carbonate of soda and stir into the bouillon, just enough to turn red litmus paper slightly blue. Care must be taken not to add too much alkali, for in the preparation of agar it is almost impossible to obtain a clear filtrate.

The bouillon is now a beautiful golden color and clear. A number of tubes can be filled about one-fourth full and the balance filled into Florentine flasks for future use. The necks must be stuffed with tightly twisted absorbent cotton, and all the tubes and flasks then put into the autoclav and sterilized for thirty minutes at 240°F.; then after removing they must be kept in a dark place so that no sunlight shall strike them. As mentioned in last chapter, strong sunlight will cause the formation of dioxygen, formaldehyde and traces of other antiseptics, through the oxidation of sugars and fats.

Dextrose and Milk Sugar Bouillon may be made by adding 2 per cent. of either to the bouillon. This combination is used to demonstrate biological peculiarities of various bacteria towards sugar. In the same manner and for the same purpose, Glycerin Bouillon is made by adding 4 per cent. glycerin to ordinary bouillon. Carbolic acid is added to bouillon; 6 per cent. of a 5 per cent. solution of carbolic acid will retard the growth of undesired bacteria, and is used in the isolation of certain germs found growing with very many different species, which under ordinary circumstances grow too luxuriantly, and crowd out the species sought.

SOLID NUTRIENT MEDIA.

To Dr. Koch of Berlin belongs the credit of this valuable discovery: that nutrient bases could be solidified in gelatin and agar-agar, and that bacteria, when mixed throughout the mass, would grow and form colonies of their own kind, and would not extend very far (if sufficiently separated) to become mixed. By means of this class of media nearly all pure cultures are obtained. If Pasteur had taken advantage of this valuable method in his time, a great part of his labors would have been made easy, and the science of bacteriology would have made greater progress than it has, although wonderful discoveries have been made, yet the possibilities are almost unlimited. Outside of electricity, we believe that there is no science which has the possibilities that lie within the range of the bacteriolgist. There are a number of diseases, the cause of which is still mysterious, there being no definite organism known to be responsible; there are smallpox, scarlatina, yellow fever, foot and mouth diseases, rhinderpest, syphilis, and many other diseases which have not been traced positively to specific micro-organisms, and there are a number of cases of spoilage in foodstuffs which have never been investigated, so that we cannot help regretting that Pasteur did not give us at least twenty years advancement by using solid nutrient media in his time. We owe to this great genius much of our present knowledge, but how much more he might have discovered with solid nutrient media to facilitate his labor is only a matter of speculation.

GELATIN MEDIA. Make the same quantity of bouillon as previously given, leaving out the peptone and salt; pour over this 100 grammes of fine gelatin, 10 grammes of peptone, and 5 grammes of common salt. Dissolve these by placing pan in boiling water, and after they are thoroughly mixed make slightly alkaline, as directed under "bouillon." Place the solution in the filtering apparatus (Novy's), attach suction pump and filter, or filter through four thicknesses of flannel if you have no other means. The liquid is then poured into tubes and Florentine flasks stoppered with cotton and sterilized at 212°F. for one hour on three successive days. If it is desired, Petri dishes may be placed in the autoclav and sterilized at the same time, and these may be filled with the gelatin medium. When sterilizing Petri dishes I have found it wise to turn both tops and bottoms upside down in wire basket, which prevents the accumulation of condensed water, so undesirable. The gelatin will harden if placed in a temperature below 75°F., so the best method is to place it in a refrigerator. Since gelatin melts at about 75°F., it cannot be used advantageously in hot weather, but in cold weather is advantageous, because it is very clear, is easily prepared, and the colonies of bacteria grown on it have more characteristic peculiarities than on agar.

Special preparations may be made with gelatin by adding grape sugar, milk sugar, glycerin or carbolic acid, as directed under "bouillon."

Solid culture media can be made by using gelatin with the juices of fruits, vegetables and the liquid or special food preparations. Usually 10 per cent. gelatin will be found sufficient.

These special gelatin preparations have great value in the study of food spoilage, because the organisms in pure cultures will produce the very changes so often observed in spoiled canned goods, while in the regular meat juice gelatin these special characteristics may not be revealed.

For the cultivation of the heat loving bacteria, the lactic group, and many pathogenic species, gelatin is not very satisfactory, since it cannot be incubated, but for the demonstration of liquefying bacteria it is fine, and furnishes means of differentiating species, where agar and even blood serum fail to reveal this characteristic.

AGAR-AGAR MEDIA.

AGAR-AGAR is prepared from a seaweed which grows on the coast of China and Japan, and is used in the place of gelatin, over which it has many advantages. It is more difficult to prepare, however, and considerable time and care must be given it to produce a good clear medium. *Formula*: The meat juice is prepared as directed under the head of "Bouillon," and 10 grammes of peptone and 5 grammes of salt are added, as directed under "Gelatin," but instead of gelatin 1 to 2 per cent. agar is used. The agar is cut up quite fine, and thoroughly washed after being weighed. Place the pan in the autoclav and slowly raise the temperature to 240°F., maintaining same for about 10 minutes; then lower the pressure and filter the fluid through Novy's agar apparatus, having previously made the liquor slightly alkaline with sodium carbonate solution, using great care not to add too much to avoid cloudiness. Novy's apparatus gives a clear, golden filtrate, which is beautifully transparent after solidification. Flasks and tubes are filled as directed under "Gelatin," and these, with the desired number of Petri dishes, are placed in the autoclav and sterilized at 240°F. for 35 minutes. The agar does not solidify until the temperature falls to 102°F., and the tubes may be slanted by laying them down so that the liquid agar will flow up towards the cotton pretty well; by raising the mouth of the tube, any desired slant may be obtained. The Petri dishes are filled while the agar is still hot. There is always considerable condensation water left on the surface of solidified agar, and to minimize this the temperature of the dishes should be about the same as that of the agar when it is poured out. After agar has solidified it does not melt again except at high temperature,

thus it has the advantage over gelatin, in that it may be kept at blood temperature in the incubator. There are a number of bacteria which require this temperature for characteristic growth. Agar is the best medium for growing motile bacteria which are to be stained to demonstrate their flagella. Bacteria, when grown on gelatin or in fluids, carry with them so much of the medium upon which they are growing that the flagella cannot be stained properly.

Various combinations of agar are made for special cultures, such as those described under "Gelatin." Chemicals, such as saccharate of iron or tartrate of iron, may be added to agar to demonstrate the production of sulphuretted hydrogen by certain species of bacteria; lactose and sterilized litmus tincture are sometimes added to demonstrate the ability of certain species to cause fermentation of lactose and the production of acids. Agar may be streaked with sterilized blood to cultivate special bacteria. In fact agar is the best nutrient medium for general purposes.

POTATO MEDIA. The growth of many species of bacteria on potato is often of great value in assisting the bacteriologist to identify them. Owing to the very resistant forms of germ life found naturally on potatoes, it is difficult to prepare sterile media. There are three varieties of bacteria found growing on the surface which will withstand considerable boiling to destroy the spores; they are Mesentericus vulgatus, Mesentericus fuscus and Mesentericus ruber, the spores of the latter being able to withstand six to ten hours' boiling. To prepare potatoes, they must be thoroughly washed in water and the eyes removed, then sterilized in the autoclav for fifteen minutes at 240°F. Before they are removed, the hands should be washed in a bi-chlorid of mercury solution, 1 to 1,000, and a sterilized knife may be used to cut the potatoes in halves lengthwise. Place the pieces, cut side up, in culture dishes having filter paper in the bottoms. The filter paper should be wet with the mercury solution, to prevent contamination. The surface of the potato may then be streaked with the pure culture of the bacteria. This work may be simplified by cutting the potatoes into slices and sterilizing in autoclav for twenty minutes at 250°F., and then transferring into sterile Petri dishes.

Potato juice may be combined with agar 1½ per cent., or gelatin 10 per cent., and a small quantity of 1 per cent. solution iodide of potassium, and sterilized thirty minutes at 240°F. This is an excellent medium for growing the typhoid bacilli, because it is slightly acid, and the bacilli form threads which have a beautiful serpentine movement.

MILK MEDIA. Milk is used to demonstrate the power of coagulation of certain bacteria, also to show whether they produce acids or alkalis, or whether their action is amphoteric. Milk is difficult to sterilize, owing to the presence of spore-bearing bacteria,

which are very resistant to heat. Too much heat changes the chemical composition of milk, so that when boiled at 212°F., citrate of lime is deposited or precipitated, and there are formed such antiseptics as formaldehyde and peroxid of hydrogen. The milk fresh from the cow, taken under aseptic precautions, is best, because it may thus be obtained almost free from bacteria. Fill the milk into sterile tubes, plug them with cotton, and heat to 160°F. for one hour for five successive days, keeping same always in a temperature not to exceed 70°F.

Tincture of litmus added to milk until slightly blue, before sterilizing, is very useful in determining the acid producing power of certain species of bacteria. The formation of acid will change the color to pink or red.

For most purposes, milk put into test tubes and sterilized for fifty minutes at 250°F., will prove satisfactory; the chemical alterations are not so great as to interfere with the study of non-pathogenic bacteria. Milk is not a good medium for growing bacteria, except for the purpose indicated, because it is not transparent and if solidified by adding gelatin or agar and used for plate cultures, the colonies of bacteria are very hard to find. The milk may be coagulated, however, and the clear fluid used with peptone and salt and solidified with 10 per cent. gelatin, or 2 per cent. agar, and have value in the cultivation of bacteria associated with milk.

BLOOD SERUM. This nutrient medium is valuable for growing various germs which produce ptomaines and toxins. The power of liquefying solidified serum is useful for identification of species. The blood is taken from the animal under aseptic precautions and allowed to stand one day in refrigerator, when the serum may be drawn off and filled into tubes. The serum will be sterile if due care has been exercised. Tubes of serum may be sterilized in Koch's blood serum sterilizer one hour at 150°F. for five successive days. They may be solidified in Koch's apparatus for solidifying blood serum by heating to 170°F.

BREAD MEDIA FOR CULTIVATING MOLDS. Bread, when made moist, is acid in reaction, and is a nutrient medium for molds; bacteria do not grow well on acid media, so the isolation of various species of Hyphomycetes is made easier, because acid is favorable for their growth. Fine bread crumbs are collected in the bottoms of several test tubes and sterilized water is added, sufficient to make a paste. The tubes are sterilized by boiling for three successive days at 212°F. for fifteen minutes.

METHOD OF MAKING CULTURES.

In order to determine the different species of bacteria which are causing spoilage or disease, it is necessary to separate them one from another and study them in pure cultures. It rarely happens

that one species is found alone; there are usually various kinds of bacteria growing together, and to separate them requires careful manipulation, which is easily accomplished by practice, and aseptic precautions. If we desire to cultivate the various bacteria found in a can of sour goods, we proceed as follows: A bunsen flame is forced down on to the surface of the tin, and a sterilized awl is put into the flame and pushed through the tin; if any air is sucked into the can by a vacuum, it is sterilized in the flame which covers the hole; a long platinum looped wire is then heated to incandescence and put down into the can through the hole and quickly withdrawn, and the loopful of material is transferred to liquefied gelatin and agar tubes and dishes, previously prepared as follows: The gelatin tubes are liquefied in warm water, and after singeing the cotton plug in the flame, tube No. 1 is inoculated by holding tube in slanting position in left hand, removing cotton plug with little finger of right hand, and then rubbing up the loopful of material on one side of the tube, mixing it with the gelatin; the plug is then put in and the gelatin mixed thoroughly by rolling and gentle shaking. The platinum loop is then sterilized in flame and tube No. 2 is inoculated from No. 1, both being held in a slanting position in left hand and the plugs of each removed in succession with little finger of right hand. Two or three loopfuls of gelatin from No. 1 are transferred to No. 2, and the plugs replaced; the gelatin in No. 2 is then shaken, the platinum loop sterilized as before, and in the same manner tube No. 3 is inoculated with two or three loopfuls from No. 2. After shaking and mixing thoroughly, all three are poured into sterilized Petri dishes and placed in refrigerator or on ice to solidify the gelatin, after which they are maintained at about 70°F.

In like manner a number of tubes are inoculated and placed in the anaerobic culture apparatus after chilling. It usually happens that No. 1 and No. 2 have too many colonies, which cannot successfully be separated before they grow together, but No. 3 usually contains but few colonies, which may be studied carefully as to color, shape, size and border, also the liquefaction of gelatin, if any occurs. Observations must be made and noted of their appearance in natural size, then of their appearance under various magnifications.

Agar plate or Petri dish cultures are made in the same manner, except that the agar is melted first in autoclav at 240°F., and then poured into tubes. The agar solidifies at 102°F., so this must be borne in mind and the work must be done quickly, at about 120°F.

Gelatin is difficult to manipulate in hot weather, and cannot be incubated, so it is advisable to make cultures on agar as well as gelatin. The writer has been successful in isolating the various

species in Petri dishes alone, without using the tubes, as follows: The agar is liquefied and poured into the sterilized dishes, and just before it begins to harden transfers are made by mixing a loopful of the suspected material in dish No. 1, using a long platinum looped wire and holding the lid of the dish up from the edge just high enough to permit thorough mixing. By a gentle swaying motion the mixing can be made uniform. Transfers are then made to a second and a third dish, as described in method of inoculating tubes, the platinum loop being sterilized between each transfer. Dish No. 1 will usually have too many colonies, which will grow together before they are old enough to show special characteristics; No. 2 may be better, but No. 3 will probably contain but few colonies, which may be carefully studied and transfers made to new dishes, which are streaked by pushing a sterilized platinum loop into a colony (or if the colony is very small, a needle is better), and the surface of the new dish is streaked by simply drawing the wire over the surface. Some care is required in handling Petri dishes; if there be any water of condensation either on the surface of the agar or on the under side of the cover, it should be set to one side and not used until this evaporates. Freshly made agar usually has considerable water of condensation and it is well to use only that which has stood in flasks long enough to show only slight traces, although dishes may be filled with fresh agar and allowed to stand until the surface is dry and no drops of water are visible on the under surface of the cover.

When material under investigation contains only a few scattered bacteria, which may be ascertained by examination of hanging drops, Petri dishes may be streaked without the necessity of liquefying the agar; the loop is sterilized and plunged into the material and the surface of several dishes streaked without sterilizing the platinum during the inoculations. The first one or two may not be freely distributed, but others will contain colonies here and there which are pure cultures, and these may be transferred to fresh dishes. Pure cultures will remain alive for months, and in some cases for years, in test tubes sealed and protected with rubber caps, or if plugged with sterilized cotton and sealed with wax (having previously moistened the surface of the cotton with corrosive sublimate.) Mold destroys many cultures; it starts to grow in the cotton and will often grow through it, down along the inside of the tube for several inches, until it reaches the nutrient material. It is well to make fresh transfers of cultures which are desired to be kept, at least once in three weeks.

As it is necessary to cultivate bacteria in the absence of oxygen, a special apparatus is desirable, one employed for such purpose (by Novy) is worthy of special mention, since it may be used with

hydrogen or the pyrogallate method for both tube and plate cultures.

For very fine work, hydrogen is preferable to any other gas for anaerobic cultures, for the reason that other gases permeate the culture media to a certain extent and have some influence on the growth of delicate organisms. The cost and trouble connected with this method are too great for ordinary work, so the pyrogallate method is generally employed, which may be described as follows: The test tubes are put into apparatus in upright position on a

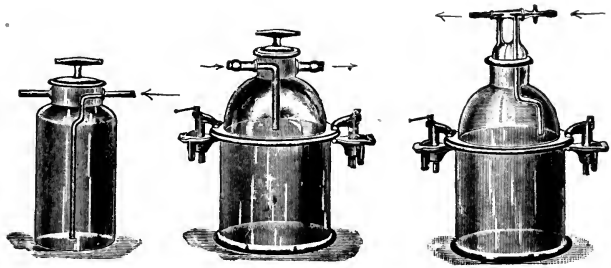


Fig. 28

wire rest, which fits in glass cylinder about two inches above the bottom; on this wire the Petri dishes may be placed also; before sealing, a quantity of pyrogallic acid and a 1-16 dilution of normal potassium hydroxid, or caustic potash, are put into the bottom of the apparatus so that the liquid will be a litle over one inch deep. The pyrogallate formula is, one part pyrogallic acid and ten parts of a 1-16 normal caustic potash solution. (A normal solution contains about seven grammes to 125 cubic centimeters of water.) The apparatus must be sealed quickly, and the glass stop-cock at the top shut off, so that there will be a complete absorption of the oxygen within the chamber. The apparatus may be placed in the incubator or may be left at room temperature, according to the nature of the organisms under investigation. In this manner it may be demonstrated if a given species of bacteria is strictly anaerobic or whether it is facultative anaerobic.

In canned goods which have soured, there are frequently found strictly anaerobic bacteria growing along with other species which are aerobic by nature. The can contains oxygen after every sterilizing process, and this oxygen is used up by the aerobic bacteria, and then the anaerobic varieties begin to grow, where the sterilization has not been complete. This is a common phenomenon and explains our failures sometimes in isolating living bacteria from cans of sour goods, where the anaerobic apparatus is not employed. In the natural putrefactive processes going on in the free atmos-

phere the anaerobic bacteria depend upon the aerobes to use up all the oxygen, while they remain dormant until there is a favorable condition of environment for their growth.

STAINING.—When living bacteria are viewed through the microscope, in order to see them well, a 1-12 oil immersion lens must be used and the result is not very satisfactory so far as the size and general characteristics are concerned. If the bacteria are motile this may be learned, but it is hard to follow an actively motile bacillus, for it is in focus usually only an instant, and then it is necessary to use the fine adjustment to bring it into focus again, which often fails. Non-motile organisms are clumped usually, but owing to the great transparency of bacteria the study of the living unstained germs is not very satisfactory.

The art of staining has enabled the bacteriologist to determine in many instances the kind and class. Many bacteria have peculiarities in their staining properties, and these peculiarities help the student. There are certain staining methods which fail with some species and the staining of flagella often aids the worker in determining the species by the number and character of the flagella of motile organisms. There are some motile bacteria which seem to possess no flagella, and it is thought that their motility is achieved by an undulating membrane attached to them, (I have never seen any motile bacilli without flagella), and the absence of flagella from the cell of a motile organism can be ascertained only by negative results in staining. Some bacteria are surrounded by a capsule, which is brought out by staining. Spores are easily located within the cells by their resistance to staining; the cell stains well, but leaves the bright spore almost transparent within the cell membrane. The spores themselves are stained by special methods and the rest of the rod may be stained with a different colored dye, which produces a beautiful effect in permanent mounts.

The first step in staining is to have clean cover-glasses (cover-glasses are either round or square pieces of glass only about 1-100 to 1-200 of an inch thick,) which are sold in small boxes holding half an ounce. The cover-glasses are covered usually with a fatty substance, which is removed with difficulty. This fat is removed by soaking a number in sulphuric acid for a day or two, then washing with caustic soda or potash, and then further cleaned by a mixture of alcohol and ammonia. (See also method of cleaning cover-glasses described under flagella staining). It is well to keep cover-glasses in absolute alcohol in a bottle with ground glass stopper to avoid evaporation. Just before using they are lifted out of the alcohol one at a time, and dried with clean linen free from fat, then passed quickly through a flame. If allowed to get too hot the edges melt down or the shape changes either concave or convex, which is undesirable, or the glass flies all to pieces, which

is often the case, but may be overcome by quick work. A small drop of distilled water is placed in the center of the glass, and a small speck of material containing germs is mixed with the drop, care being taken not to have too many germs; the drop is then evenly spread over the whole surface, and if the glass be clean this will be done easily. Should the fluid collect in small droplets, it indicates that the cover-glass is not clean, and the process of cleaning the cover-glass must be gone over again so that the fluid will spread evenly over the entire surface. For convenience in handling, the cover-glass is held in a small forceps. (One devised by Novy for this purpose is very good.) The fluid is allowed to dry onto the glass by evaporation, or may be hastened by waving in the air. When absolutely dry it is fixed by passing through the Bunsen flame three times quickly, keeping specimen side away from actual contact with the flame. The bacteria are thus firmly fixed on the glass, so that they will not wash away when the staining is done. A small drop of water is then placed on the specimen side, and allowed to spread over the surface, and then the glass is flooded with an aqueous solution of dye, such as carbol fuchsin, methylene blue, gentian violet, Bismarck brown, etc. The glass holding the rounded drop of color is held about three inches over the flame and heated until vapor is seen to rise, and this is maintained for several minutes, care being exercised to avoid actual ebullition. It is not a good plan to force the staining by too much heat; the best results are obtained by gentle heating for a longer time, and if the stain is allowed to cool before using water to wash excess away, the danger of cell shrinkage is minimized. After washing off the excess of stain, a few drops of diluted alcohol will clear up the field, but it must be washed off at once; then take a clean glass slide and place the cover-glasses on it, specimen side down, removing excess of water by filter paper, and dry the upper surface; now place a small drop of cedar oil on center of cover-glass; put slide under microscope; bring down 1-12 objective until it touches the oil and bring into focus with fine adjustment. If the specimen is all right the cover-glass may be floated off by water and allowed to dry in air or by touching edge to filter paper, waving in air, etc., and when dry it may be cleared by flooding both sides with xylol, then turned edge down on filter paper and finally held about twelve inches above flame until dry. To mount cover-glass, take a clean slide, warm over flame until all moisture is forced away, then place a small drop of Canada balsam dissolved in xylol in the center. (Xylol balsam is put up in tubes all prepared for use. A small drop may be squeezed out of the tube onto the slide, experience teaching just the required amount.) The slide is again held over flame to drive away all mosture, and the cover-glass is also warmed and placed, specimen side down, upon

the drop of balsam, and may be pressed down firmly by laying a sheet of filter paper over it, or by using a cork of the same diameter. There should be only enough balsam to fill up space, but it often happens that some excess will be squeezed out; this will harden eventually and will cause no inconvenience unless it is too excessive, in which case it may be removed with a little xylol and clean linen.

Many microscopists have trouble in obtaining clear work on account of moisture on the slide or cover-glass during the mounting, so I wish to call particular attention to the perfect drying of both slide and cover-glass before using the xylol for clearing.

METHOD OF OBTAINING AND STAINING CONTACT SPECIMENS.—This method is used to show the "Swarming Islands" of such bacteria as Proteus Vulgaris, Proteus Mirabilis and Proteus Zenkeri, which are shown in plates.

The colonies are grown on gelatin, and when the bacilli begin to swarm and branch off from the parent colony a cover-glass is dropped carefully over a colony and gently pressed; it is then lifted straight up, avoiding any lateral movement, and dried in the air, then stained as directed in the ordinary method. If the colonies show liquefaction, contact specimens cannot be made.

GRAM'S METHOD OF STAINING.

This method of staining is used to differentiate the species. There are a great many bacteria which do not retain the stain, while others having great resemblance take the stain readily. The age of the culture and the medium upon which it grows have something to do with the results.

METHOD.

1. The cover-glass specimen is stained for a few minutes with Ehrlich's anilin-water gentian violet. (Anilin oil=4 c.c.+water 100 c.c.+11 c.c. of concentrated alcohol solution of gentian violet.)
2. Wash with water and use Gram's solution of iodin (iodin crystals 1 gramme+iodide of potash 2 grammes+water 300 c.c.) until the stained surface blackens, which requires about half a minute.
3. Wash with alcohol until excess color is removed. Then the specimen may be examined under the microscope to ascertain if the bacteria have taken the stain.

METHOD OF STAINING TUBERCLE BACILLI.

The tubercle bacilli are found frequently, and often in large numbers, in fresh milk and also in butter. The method of staining here given is used to demonstrate the bacilli from phthisical pati-

ents and is applied to the sputum, which is carefully spread over the surface of a cover-glass, air dried and fixed in flame as in ordinary method. Tubercle sputum is easily obtained, and the staining of the bacilli affords excellent practice for the beginner.

For examining milk and butter a centrifugal machine is used to obtain a sediment, which is more apt to show the presence of consumption germs than a small quantity taken at random.

The suspected milk is put into the bottles and the machine is used for a few minutes, the fluid is poured off and the cover-glass is spread with some of the sediment. If butter be suspected, a small quantity is put into a test tube about three-fourths full of water, which is then heated in water to melt the fat. The tube is thoroughly shaken and put on ice to solidify the fat, after which the fluid is put into the centrifugal machine, the same as described for milk, and a cover-glass spread is made of the sediment. The cover-glass thus prepared will contain too much fat, so it must be air dried and heated slightly, and laid in a mixture of ether and alcohol (1 to 3) for a few seconds, then removed, air dried, fixed in flame and stained as follows:

The cover-glass is flooded with Ziehl-Neelsen's carbol-fuchsin (fuchsin 1 gramme+alcohol 10 c.c.+water 100 c.c.+carbolic acid 5 grammes), and heated over flame until vapor arises and set to one side. Repeat three or four times; wash off excess of stain with water and decolorize with a twenty per cent. solution of sulphuric acid and wash acid off with water. If still too red, use sulphuric acid again. When washed the specimen should be pink. The cover-glass is then flooded with Loeffler's methylene blue (concentrated alcohol solution of methylene blue 30 c.c.+watery solution of caustic potash 1:10,000—100 c.c.), and heated for a few seconds, then washed under the water tap until all excess color is removed. The tubercle bacilli will be stained a deep red, and the surrounding field will be blue, which makes a beautiful contrast.

Since one-seventh of the population of the world die from consumption, this disease germ is most interesting for study and bacteriological investigation. It is transmitted from one person to another in various ways, by breathing particles of floating matter containing the bacilli in the homes of consumptives, in public conveyances and buildings, and in articles of food, such as milk and butter. It is not hereditary. The germs are destroyed at 212°F., and there is danger only in such foods as are consumed in an uncooked state. Pasteurization destroys them in milk, and this method of treating milk and cream intended for butter-making is to be highly commended.

Bacillus Tuberculosis, Koch (1882)

TUBERCLE BACILLUS.

Origin.—In tuberculosis of mammals; in lupis vulgaris. The bacillus of chicken tuberculosis is distinct from that of mammals.

Form.—Rather long, very narrow rods, smaller than the diameter of a blood cell. Are sometimes beaded. May be straight, but more frequently are slightly bent or nicked; distinctly rounded ends. Usually single, but sometimes forms short threads of three to six cells. It is frequently found in small bunches in the sputum, tissues, etc. It rarely occurs in branching form and with club-shaped ends.

Motility.—Is not motile.

Sporulation.—A number of bright bodies are frequently seen in the cell, but cannot be considered true spores. The bacillus is resistant to heat, dessication, acids, putrefaction, etc., in a relatively high degree.

Oxygen Requirements.—It is a facultative anaerobe. Requires free access of oxygen for growth.

Temperature.—Grows best at 37-39° C. Slight variations above or below this temperature will stop the growth. Will not grow at ordinary temperature.

Behavior to Gelatin.—No growth at ordinary temperature. Does not peptonize blood-serum.

Infection.—Takes place ordinarily along the respiratory tract—Inhalation tuberculosis. It may occur through wounds—Inoculation tuberculosis, also through food—Intestinal tuberculosis. The bacilli introduced into the intestines may localize in distant parts of the body.

METHOD OF STAINING SPORES OF SPORE-BEARING BACILLI.

The spores when free, or when fully formed within the bacilli, are very difficult to stain, but by heating the cover-glass specimen five or six times with carbol-fuchsin or gentian-violet, they will take the stain. The rods will decolorize in one minute in a 3 per cent. solution of HCL alcohol, and after washing they may be stained with methylene-blue, in contrast to the spores if fuchsin was used, or if the spores were stained with violet the rods may be stained with Bismarck-brown.

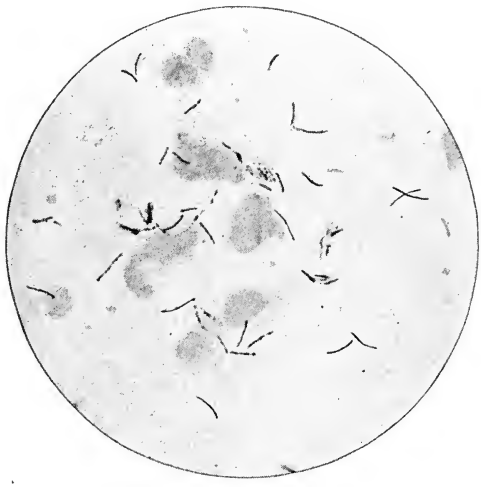


Plate 17 Tubercle Bacilli

Tubercle Bacilli. Photomicrograph from a Coverglass Specimen obtained from sputum. Mag. X 1,000.

*THE DEMONSTRATION OF THE FLAGELLA OF MOTILE BACTERIA AND A SIMPLE METHOD OF MAKING PHOTOMICROGRAPHS.

Methods Worked Out by the Author.

Motile bacteria should be represented in illustrations just as they are naturally. The photomicrographs usually displayed in works on bacteriology do not as a rule represent motile bacteria as they should be. I, therefore, took up the study of staining these organisms and endeavored to discover a method which would give good results with all kinds of bacteria and I made a comparative study.

I have always obtained the best results with all species of bacteria, excepting the anaerobic, by streaking the surface of 2 per cent. agar in Petri dishes, and the streaked culture should always

*From my address delivered before the Society of American Bacteriologists, at Philadelphia, Dec. 27, 1904.

be made from the young growth in bouillon and never from an agar or gelatin transfer. I usually inoculate a tube of bouillon the night before and streak the surface of the agar early on the following morning and then place the dishes in optimum temperature, so that I may get the most rapid growth possible. I find that 2 per cent. agar is preferable to that of less per cent., because the bacteria as a rule do not collect much debris from the culture media. Bacteria differ widely in the number and character of their flagella. Some are peritrichous, having large numbers growing out from all parts of the cell; some are lophotrichous, having a bunch of

Plate 18 Typhoid Bacilli, Flagellated

Magnified 1,000 diameters.

flagella at one end; some are amphitrichous, having a flagellum at either end; some are monotrichous, having a single terminal or polar flagellum. The flagella of different organisms vary in character; some are extremely fine, so delicate that they stain with difficulty; some are long and wavy; others are short and may be almost straight. The anaerobic bacteria possess flagella which are extremely curly, and it is possible to determine from the character of its flagella whether an organism is an anaerobe or an aerobe. Many motile bacteria produce spiral bodies which are termed "Giant Whips" by Novy; some of these will reach 100μ in length.

I found that the methods for flagella staining described by the old authors had to be modified considerably in order to get good results. By constant practice and very hard work, often prolonged into the small hours of the morning, I finally succeeded in my ef-

forts. I found there were six general classes of bacteria, each different from the other, in its manner of growth, making it necessary to treat each class in a different manner. I divided the motile bacteria into six classes for staining purposes:

FIRST—bacilli which grow like the streaked culture of the Typhoid, such as Typhoid and Colon.

SECOND—bacilli which produce wrinkled or folded growths, such as Mesentericus fuscus.

THIRD—bacilli which send out a thin, almost transparent growth over the surface of the agar, such as Bacillus Subtilis and Bacillus Megatherium.

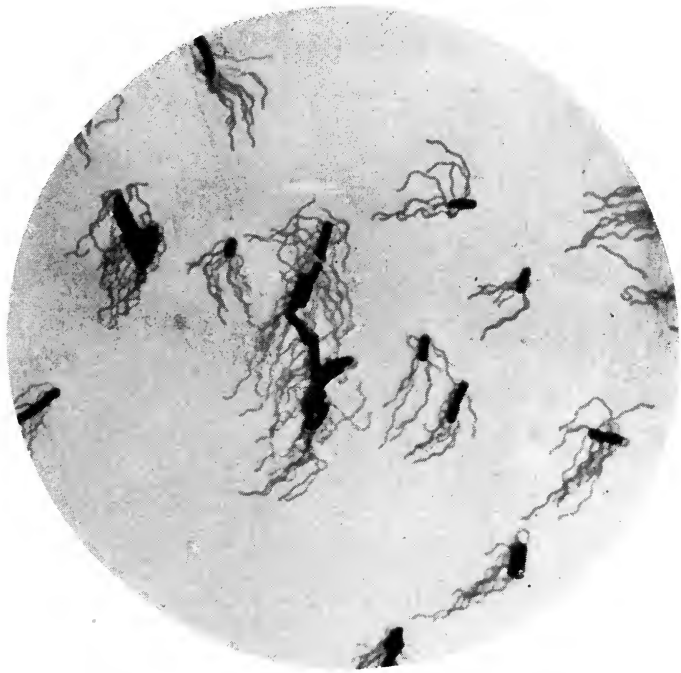


Plate 19 Bacillus Mesentericus Fuscus, Flagellated

Magnified 1,500 diameters.

FOURTH—bacilli which produce slime, such as Bacillus Vulgatus and Bacillus Viscosus.

FIFTH—bacilli which produce pigments, such as Bacillus Prodigiosus and Bacillus Cyanogenus.

SIXTH—anaerobic bacteria, such as Bacillus Tetanus, Oedema and Symptomatic Anthrax, etc.

MANNER OF MAKING SUSPENSIONS IN WATER.

(1) Bacteria resembling Typhoid streak cultures have very young and actively motile bacteria on the periphery of the growth. From this the material is taken and transferred to a large drop or two of distilled water which has previously been boiled. The platinum loop should be made from very fine platinum wire, only about half the size of the loop used for general purposes. This fine loop will gather sufficient material without taking up any of the agar. The material usually clings tenaciously to the loop, but may be liberated by the aid of another platinum wire, if care is exercised. The bacteria are then allowed to disseminate spontaneously throughout the drop of water, so that the finest specimens will swim to the outer edges from which the cover-glass preparation is made. Bacteria which have few flagella and those whose flagella have been broken will remain near the center.

(2) Preparations made from bacteria which produce wrinkled or folded growth are made before the wrinkled growth is formed. In order to get a good preparation from this group of bacteria the agar should be streaked in the morning and then carefully watched for the first appearance of growth and from this a satisfactory preparation can be made.

(3) The thin, transparent, spreading growth is one of the best for demonstrating flagella. This growth is almost invisible and is composed of very young and actively motile bacteria. In order to get a good preparation from this a curved platinum wire is used to gently collect the bacteria *en masse* and then the small loop is employed to make transfers to the distilled water.

(4) The slime-producing bacteria are very difficult for the demonstration of flagella. The slime collects between the flagella and the mordant fixes the slime as well as the flagella, so that the stain completely covers the delicate organs of locomotion. I found that this slime could be precipitated by shaking a water suspension with chloroform. A very young growth of the organism is used and transfers are made to about 1 c.c. of distilled water in the test tube until the water is made very cloudy. The slime increases the cloudiness and this is necessary in order to have a sufficient number of bacteria to make a fine preparation. This cloudy suspension is then shaken with chloroform, which seems to cut away the slime from between the flagella; then the cover-glass preparation is made from the water above the chloroform.

(5) Bacteria which produce pigments soluble in chloroform are treated in the same manner. Those whose pigments are soluble in water and not in chloroform are more difficult to stain. I usually hold the cover-glass under the tap after fixing the preparation in

Plate 20 Bacillus Subtilis, Flagellated

Magnified 1,000 diameters.

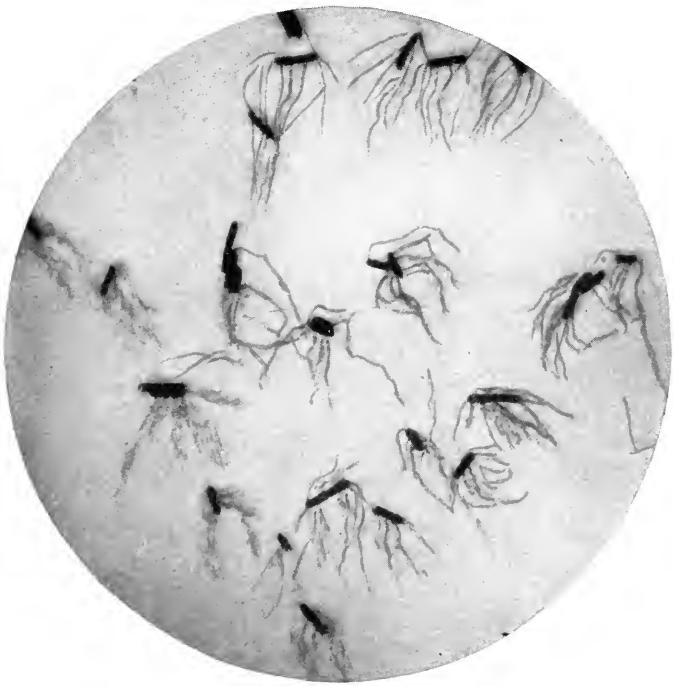


Plate 21 Bacillus Mesentericus Vulgatas, Flagellated

Magnified 1,200 diameters.

the flame previous to adding the mordant. In this way much of the soluble pigment is removed.

(6) Good suspensions of anaerobic bacteria are the most difficult of all to obtain. Bacteria which are imbedded in stab cultures do not make good preparations, because the agar and debris cling tenaciously to the flagella. It is extremely difficult to get a good surface growth of obligative anaerobes, because it usually requires two or three drops of a young bouillon culture for surface inoculation and when the growth appears the surface is covered with the old, partially dissolved cells and free spores. Still, some very fine preparations can be made from the surface of the culture. The best results are obtained as follows: The medium is 2 per cent. glucose agar in slants and the inoculation is made back of the slant between the agar and the wall of the tube. I slide the needle down back of the slant and let it fall forward; I introduce two or three drops of a young bouillon culture and then replace the agar. By excluding oxygen and maintaining a blood temperature for thirty-six hours, a fine growth of bacteria usually appears between the agar and the wall of the tube and beautiful preparations can be made from this. Many rods containing spores still retain a full equipment of flagella.

CLEANING THE COVER-GLASSES.

I prefer the No. 1 round cover-glass, which when new are covered with a thick, greasy substance quite difficult to remove. Cover-glasses used for the demonstration of flagella must be absolutely clean, and this is a most important feature. For removing the grease they are covered with suphuric acid and allowed to stand for one day. The sulphuric acid is poured off and they are then covered with bichromate of potassium and allowed to remain in this for several hours. This acid is then poured off and the cover-glasses are washed with distilled water and transferred to a jar containing absolute alcohol, where they remain until ready for use. A single cover-glass is removed with clean forceps from the alcohol, transferred to a piece of clean, well-washed linen and dried without touching it with the fingers. The cover-glass is then taken in the forceps and passed several times through the Bunsen flame, so that every particle of fat or grease is removed, and it must appear clear and free from blemishes. Many cover-glasses are lost after heating in the flame, particularly if there are any currents of cold air through the room, but since the perfect condition of the cover-glass is so important the loss of two or three is immaterial.

Bacillus Oedematis Maligni, No. 2, Novy (1893)

Origin.—Obtained from guinea-pigs which had been inoculated with milk nuclein obtained from casein by digestion with artificial gastric juice.

Form.—In the animal body it is usually found in single rods, four to five times as long as wide; also occurs in short threads. On artificial media the rods are straight or bent; peculiarly twisted threads are sometimes formed. The contents are frequently granular, showing a bright body at one end.

Motility.—A slight, swaying motion, which is not always present. Possesses lateral flagella, and gives rise to giant whips 40 to 72 microns long in pure cultures as well as in the animal.

Sporulation.—Has not been observed.

Anilin Dyes.—Stain readily. Gram's method may be used.

Growth.—Depends upon vitality. Grows rapidly when taken from an animal.

Plates.—On glucose agar at 37° good colonies will develop in two or three days; these have irregular, fibrillated border, and frequently develop gas bubbles. Giant whips are sometimes found.

Stab Culture.—Grows only in the lower part of the tube. In glucose agar properly alkaline, a distinctly visible growth develops along the line of inoculation; gas is produced which soon tears apart the agar. The cultures soon die out.

Streak Culture.—Grows on glucose agar only when oxygen is completely excluded; grows in the form of a white film, spreading over the surface. Involution forms develop on acid agar.

Bouillon.—A fine growth is developed which settles to the bottom as a loose, flocculent sediment in twenty-four hours; the liquid above becoming clear.

Glucose Gelatin colored with litmus.—Liquefies and produces acid. The litmus is reduced and turned red.

Oxygen Requirements.—It is an obligative anaerobe. Will grow in vacuum hydrogen, nitrogen, carbonic acid and illuminating gas.

Temperature.—Does not grow below 25° C. Grows best at about 39° Will withstand freezing for twenty-four hours.

Behavior to Gelatin.—Liquefies.

Aerogenesis.—Produces gases in alkaline media. Forms volatile acids. as butyric acids, etc., in artificial cultures and also in the body of rabbits.

Attenuation.—Cultures lose their virulence when exposed to light or left in hydrogen. Can be kept in the dark or by passing through animals. Lost virulence may be restored by inoculation with a mixed culture containing Proteus vulgaris.

Immunity.—Is not produced by non-fatal inoculation, or by old weakened cultures, or by the serous exudate of the pleural cavity.

Pathogenesis.—Subcutaneous injection of ¼ c. c. of hydrogen bouillon cultures will kill guinea-pigs, white rats, white mice, rabbits or doves, in twelve to twenty-four hours. Marked subcutaneous edema are present. Serous exudates in thoracic and abdominal cavities. Cover-glass preparations made from subcutaneous tissue or serous surfaces usually show very large numbers of bacilli; giant whips are also frequently present, being visible as colorless spirals.

Diagnosis.—It is readily distinguished from symptomatic anthrax and malignant edema by morphological characteristics.

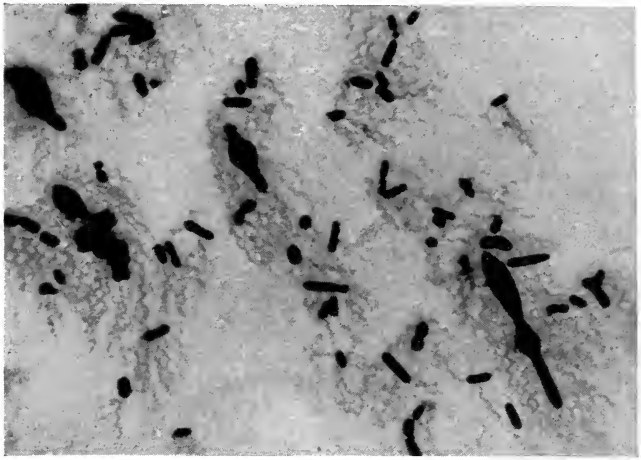


Plate 22 Bacillus Tetanus, Flagellated

Magnified 1,200 diameters.

Plate 23 Bacillus of Malignant Oedema

Magnified 1,500 diameters.

PREPARATION OF THE STAINING AGENTS.

The fixing agent is mordant and the stain is carbol gentian violet or preferably carbol fuchsine.

THE MORDANT.

2 grams dessicated tannic acid.
5 grams cold saturated solution ferrous sulphate (aqueous).
15 c.c. distilled water.
1 c.c. saturated alcoholic solution of fuchsine.

The tannic acid is dissolved in the water first, by the application of gentle heat; then the ferrous sulphate and then the alcoholic solution of fuchsine are added.

To these ingredients, I have always found it advisable to add a certain amount of sodium hydroxid, a 1 per cent. solution, varying from ½ to 1 c.c. The best grade of filter paper is used for filtering the mordant, and there should be left a heavy precipitate. After filtering, the color of this mordant should be of a reddish-brown hue, not clear, but somewhat cloudy, and this mordant must be used within five hours after it is made. After that time, it loses its staining power. This is indicated by its gradual clarification and darkened color. It gives the best results when strictly fresh, and accomplishes its work in a much shorter time, so that very little if any heating is required when it is placed on the cover-glass preparation.

CARBOL FUCHSINE.

Take about one gram of granulated fuchsine (not the acid fuchsine), put it in a bottle, and pour over it about 25 c.c. of warm absolute alcohol. Shake vigorously, and let it stand for several hours before using. The carbol fuchsine is made by diluting the saturated alcoholic solution four or five times with a 5 per cent. solution of carbolic acid. Carbol fuchsine should be freshly made, heated and filtered before using.

Every organism differs from other organisms in its manner of absorbing the stain, so that some experimental work is necessary to determine just how the stain should be applied. In a general way we proceed as follows: A small loop full of the clouded water, obtained as described in the first part of this article, is transferred to the cover-glass and gently spread over as large a surface as possible. Care must be exercised in spreading the drop. I usually carry the drop around the surface without touching the glass with the loop. In this way the surface is moistened, and the loop does not tear off the flagella. A confluent spread does not give as good satisfaction as a streak spread with a small space be-

Bacillus Anthracis Symptomatici, Feser and Bollinger (1878)

SYMPTOMATIC ANTHRAX, BLACK LEG, QUARTER EVIL; CHARBON SYMPTOMATIQUE (FR.); RAUSCHBRAND (GERM.)

Origin.—Found in the subcutaneous tissue, muscles, serous exudate, etc., of symptomatic anthrax.

Form.—Rather large, narrow rods, having rounded ends; almost always single, but sometimes are found in pairs. About three times as long as wide. Involution forms are seen in old cultures—swollen at the ends or in the middle.

Motility.—Actively motile, having lateral flagella; giant whips are often found. Spore-bearing rods lose their motion eventually.

Sporulation.—Spores develop near one end, which is enlarged; they are bright and oval in form; are not formed in body until after death.

Anilin Dyes.—Stain readily. Will stain by Gram's method if a strong dye acts for some time. Spores may be readily double stained.

Growth.—Rapid; best in acid or alkaline glucose media; attended with strong butyric acid odor. Will not grow except under anaerobic conditions.

Plates.—On gelatin, irregular masses are formed surrounded by dense whorl of threads. Gelatin is liquefied. On agar, the colonies usually appear as dense masses of threads; they vary, however.

Stab Culture.—In glucose gelatin, growth takes place in the lower part of the tube; gas is produced; contents liquefied. In glucose agar, growth is energetic and gas is produced, the contents of the tube being torn into several parts. Giant whips common (Novy).

Streak Culture.—On glucose agar in hydrogen, a whitish, spreading film is formed. On blood serum, growth is good; giant whips (Loeffler).

Bouillon.—Is clouded; gas bubbles accumulate on the surface; the growth settles to the bottom after several days, forming a compact, adherent sediment; liquid above remains cloudy for several days.

Glucose Gelatin, Colored with Litmus.—Under ordinary circumstances, growth develops in incubator. The litmus is reduced, then colored a wine-red, showing formation of acid. Heavy, flocculent sediment is deposited on the bottom.

Milk.—Coagulates casein rapidly; does not invert starch. Grows on potato.

Oxygen Requirements.—It is an obligative anaerobe. Will grow in hydrogen, vacuum, carbonic acid, etc. Grows in glucose litmus gelatin in presence of air.

Temperature.—Grows best at 37-38° C. Will grow slowly at room temperature.

Behavior to Gelatin.—Liquefies.

Aerogenesis.—Produces gas with disagreeable odor; gas is inflammable, consisting of marsh-gas, hydrogen, etc.

Attentuation.—Bouillon cultures lose their virulence soon but retain vitality. Attenuation occurs at 42-43°. Dry spore-bearing material becomes attenuated when heated to 80° or 100°. Virulence may be restored by inoculating animals, at the same time injecting some lactic acid. Virulence is maintained in solid media.

Immunity.—May be obtained by inoculating small amounts of virulent germ; by intravenous injections; by injection of heated cultures, 80° or 100°; in active old cultures; filtered cultures.

Pathogenesis.—Young cattle, sheep, goats, guinea-pigs and mice are highly susceptible. The horse, ass and white rat are less susceptible. Hogs, dogs, rabbits, ordinary rats, doves, ducks and chickens are almost immune. Death is produced in twenty-four to forty-eight hours in guinea-pigs by subcutaneous injection. Extensive subcutaneous bloody edema is present. Gas is present. The muscles are dark and infiltrated.

Infection.—Occurs naturally by inoculation through deep wounds; very rarely through food. Poisoned arrows used in fishing in Norway.

Diagnosis.—It is especially a disease of cattle, and not of man. It is difficult to distinguish the bacillus from malignant edema bacillus. Inoculation of the rabbit is negative, threads are absent; tendency to involutions. It is distinguished from anthrax bacillus by form, motility, by its distribution in the body and by cultural properties.

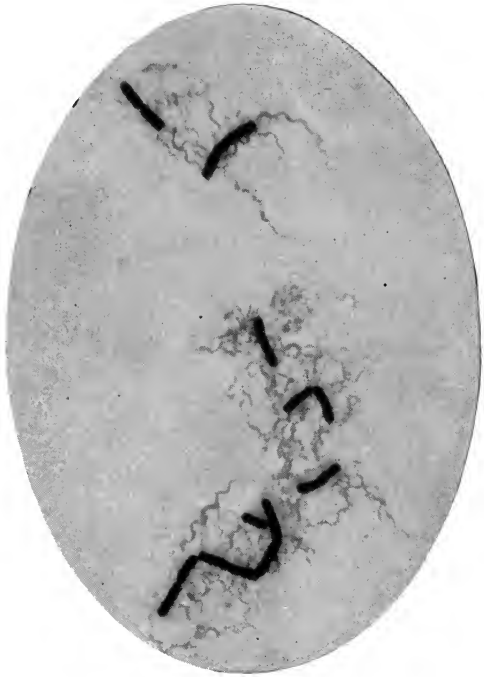


Plate 24 Bacillus of Symptomatic Anthrax, Flagellated
Magnified 1,200 diameters.

Plate 25 Asiatic Cholera, Flagellated
Magnified 2,000 diameters.

tween each streak. The finest specimens for photomicrography are obtained from the periphery of the streaks. The spreading must be done rapidly because evaporation takes place very soon. After evaporation takes place, there should appear very thin films along the track of the loop. The preparation must be fixed in the flame so that the bacteria will not wash off during the staining, but the fixing must not be accompanied by too much heating, because the delicate organs of locomotion are easily burned off. The Bunsen flame should be about one inch high; the glass held by the forceps, preparation side up, is passed down on to the flame just once and instantly removed. The cover-glass is then ready for the mordant, which is poured on, just enough to cover the surface without flowing over the edges. I find that the Cornet forceps are best suited for staining purposes, and if the cover-glass be held just a short distance within the edge, the mordant or stain will not run off. It usually happens that we are unsuccessful in demonstrating flagella, if the mordant runs off, or goes on the under side of the glass. During the steaming of the mordant, it is advisable to keep up a rotary motion in order to avoid too much precipitation. When the mordant is fresh, it requires only about one-half to one minute to get sufficient staining. The mordant is completely washed off under the tap, and this is done pretty thoroughly; a small quantity of absolute alcohol is poured onto the surafce, and this is instantly washed off. The alcohol removes a great deal of the precipitation which is found in cover-glass preparations, but considerable care should be taken to wash it off quickly and thoroughly, because it will remove the germs and flagella, if it is allowed to act only for a short time. If the alcohol is thoroughly washed off, the water is removed by holding the glass edgeways to a piece of filter paper. If the filter paper is not clean, considerable dust and fiber will be carried up on to the cover-glass, and if there is any danger of this, it is better to finally wash the cover-glass off with the distilled water and shake off the drops which cling to the glass. Then cover the surface with a carbol fuchsine or carbol gentian violet. I find in nearly all cases, that fuchsine is better than violet, and gives less precipitation, but in some cases the gentian violet brings out the flagella more prominently. This must be fresh, however, and thoroughly washed off after staining. We allow the fuchsine to stand on the cover-glass for about one-half minute, being heated just sufficiently for a thin vapor to be visible. We then heat it so that steam is given off quite freely, but never until ebullition takes place. Care must be used when heating the stain, because it is not unusual to find that there is entirely too much precipitation, and the flagella are burned off. It rarely happens that we get a cover-glass which will be stained well all over. Usually we get only certain sections where the flagella stand out prominently, and the field

is free from precipitation. Views sufficiently attractive for photomicrographing are rare. While the staining may be perfect, there will be some defects in the germs. Some may have lost part of their flagella, or there may be too much precipitation in the field, or the germs may be too close together or too scattered, and the ideal views for photomicrography are few, and it is sometimes necessary to stain up several cover-glasses, before we get a fine view. During the staining with the mordant and dye, a thin film is formed all over the glass. This must not be broken up by the application of too much alcohol, if a clear field is desired.

Some writers advocate the idea of examining the cover-glass preparations on the slide with a drop of water under the cover-glass, before finally clearing and mounting the specimen. I have been unfortunate in this procedure, and on several occasions have lost some beautiful specimens on attempting to float off the cover-glass with water, after examination. It frequently happens that the germs will stick to the slide and pull off, leaving graves surrounded by beautiful bunches of flagella, so I make it a rule to mount my cover-glass in xylol-balsam, as soon as I have finished staining. I do this as follows:

I select very thin slides, pieces of glass about 3 inches long and 1 inch wide, perfectly clear, and having no blisters. Having thoroughly cleaned the slide, I heat it over the flame to drive off moisture, and place in the center a small drop of xylol-balsam (which is Canada balsam dissolved in xylol, and comes in collapsible tubes). After thoroughly drying the water from the cover-glass after staining, I pour pure xylol all over the surface, and immediately touch the edge to clean filter paper, and then drive off the xylol with heat. It is absolutely necessary to have the cover-glass free from moisture before applying xylol. (Xylol is a refined benzine). Otherwise, a hazy appearance will be imparted to the preparation, and this spoils it for microscopical purposes. After clearing with xylol and drying, the drop of balsam is heated gently and the cover-glass, preparation side down, is pressed on to the slide so that the balsam is spread out in a thin layer between the two pieces of glass, and the preparation is, of course, thus protected from injury. The method for the demonstration of the flagella of different organisms varies, as we have said. The differences which I have noticed have been in the length of time allowed for staining with the mordant, and the fuchsine; also the amount of 1 per cent. sodium hydroxid. Great success is achieved only by careful and patient study of each organism. It is not a difficult matter to demonstrate the flagella of most motile organisms, but to get beautiful preparations is a study, and requires great care in every step of the work.

SUMMARY.

Culture to be made on 2 per cent. agar from young growth in bouillon.

Suspensions in water to be made according to nature of organism.

Cover-glasses to be absolutely clean.

Mordant to be used only when fresh.

Dye to be made fresh and used while warm.

Spread on cover-glass not to be confluent.

Fixing to be done without injury to flagella.

Staining to be done without overheating.

Washing with alcohol and water without breaking the film.

Clearing with xylol after thorough drying.

Mounting in xylol-balsam without previous examination.

A SIMPLE METHOD OF MAKING PHOTOMICROGRAPHS.

A large, cumbersome apparatus is unnecessary. The camera is about twice as long as the ordinary 4x5 camera, and the photomicrographs are taken with the camera in a horizontal position. It must be a steady apparatus and the microscope stand should be substantial and with the cone fine adjustment. Much depends upon the objective. In order to get negatives showing a flat field with clean definition I have used nearly all kinds of objectives, but have found none equal to the 1-12 oil immersion objective and No. 6 compensating eye-piece made by the Spencer Lens Co. The best plates are the isochromatic or orthochromatic swift plates, which are correct for colors. I have found the acetylene radiant preferable to gas, oil or electric light. It is slower than electric light, but brings out all details with wonderful nicety. The only screen I ever use is green glass. Printing from the negatives on glossy Velox brings out the best detail. The glossy Velox is then ferro-plated, which makes a beautiful photograph.

CHAPTER IV.

Decomposition Caused by Micro-Organisms

Decomposition Caused by Micro-organisms. Fermentation Theories. Vacuum Theory. Alcoholic Fermentation. Acetic Fermentation. Butyric Fermentation. Lactic Fermentation. Putrefaction. Reprocessing Leaks a Dangerous Proceeding.

The word fermentation is derived from the Latin word fermeo, meaning to boil. The appearance of liquids in agitation due to the vital action of micro-organisms no doubt gave rise to the word.

The word fermentation as commonly used, implies more than the processes of decomposition accomplished by bacteria, molds and yeasts. *Micro-decomposition* is perhaps a better term, since it applies directly to the breaking down processes accomplished by micro-organisms and their enzymes (products formed) only, and does not take in the chemical changes induced by chemicals, rennets and animal secretions. The term embraces also the different processes of putrefaction, which are separated by some authors, but it seems to me that they should be considered under one head.

FERMENTATION was the term that was applied to these processes by the early investigators, and the history of their labors and deductions is interesting, since it shows us the difficulties with which they were beset and permits us to see the rays of light and truth as they are let into the darkness by the different stars in the scientific world from the time of Leeuwenhoek down to the present.

The early investigators, a few excepted, fell victims to the false theory of *Spontaneous Generation.* Needham (in 1745) founded a demonstration of this theory on his failures to preserve meat juices by boiling in flasks, claiming that "infusoria" were spontaneously created from the juices themselves.

In 1765 Abbe Spallanzani took the opposite stand, claiming that if air, which had been exposed to fire, were admitted to flasks containing meat extracts, the "animalcules" would not develop. In 1836 Franz Schultz conceived the idea of filling the flasks with air filtered through sulphuric acid and potassium hydroxid, which gave him encouragement as an opponent of the spontaneous theory. The other side claimed that a chemical change in the air was made by such experiments which made it impossible for the animalcules to hatch from the vital principles of the infusions. They also

found that the methods referred to were not reliable and that micro-organisms would make their appearance in many cases. Thus the study of fermentation and its causes began to occupy the attention of investigators. In 1862 Pasteur published his researches on fermentation and Von Liebig still opposed him with the theory of spontaneous generation. Then Tyndall came forward with his absolute proof that micro-organisms did not develop from inorganic protoplasm (elementary compounds), but that they developed only as they found admittance through the atmosphere and that if infusions were sterilized fermentation could not possibly take place. By this intermittent heat process he sterilized all kinds of liquids and solid food substances and gave the opposition such a blow that the "spontaneous" theory fell.

The theory is only unproved, however, since all must admit of a beginning of all life. That the beginning of a species is due to a creative power is probably the best way of disposing of the question; at what time we cannot say; whether it is still going on we cannot say; but there is evidence that such is the case.

There are several kinds of decomposition which cannot be ascribed to the action of micro-organisms which the word fermentation would include. There is a spontaneous decomposition of sugar in vegetable and fruit cells, which when kept in a pure and uncontaminated condition, liberate carbonic acid gas CO_2, and form alcohol in appreciable quantities. This is no doubt due to the life of the fruit itself, which is living protoplasm, and when seeds are present, vital principles are therein contained which have the power to decompose the sugar in the fruit or vegetable cells. It is a curious fact that when a whole tomato is heated in a flask to the boiling point, after a lapse of time it will be found quite devoid of sugar so far as taste is concerned. Quite a liberal quantity of gas will be liberated also, and when the seeds are examined carefully the gelatinous envelope will be found perfect as before heating and the seeds are capable of germinating when planted. The decomposition takes place without the vital activity of micro-organisms. The experiment may be made by anyone interested by placing a perfectly sound ripe tomato in a thin glass jar and melting the top down to a narrow neck by means of a blow pipe, (the skin of the tomato should be washed off with a solution of bi-chlord of mercury). This narrow neck may be stuffed with sterilized cotton and the flask held over the flame just long enough to permit steam to flow freely through the cotton. A bent tube may be fastened with rubber over the neck of the flask and the end submerged in a dish of water. To measure the escaping carbon dioxid, a bottle with water is inverted over the end of the tube under water. As fast as the gas is evolved the water is expelled from the bottle, but the process is slow.

I have investigated a number of cases of so-called spring bottoms in cans of fruit, especially California fruits. Cases of canned fruit are frequently found where the bottoms of the cans spring, showing that there is no vacuum in them and quite a quantity of gas, sufficient to cause the bottom to spring out when pressed in by the hand.

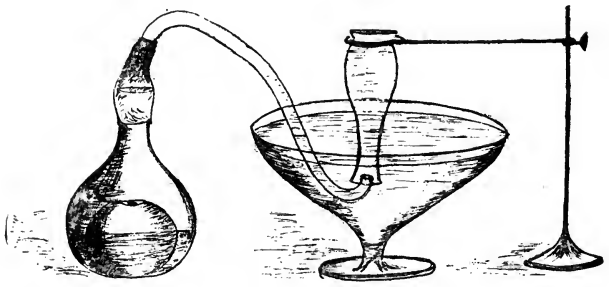


Fig. 29

In some cases I have found wild yeasts to be the cause, but more frequently the cans are quite free from bacteria or fungi of all kinds. This led me to the conclusion that there was decomposition going on from a different cause, and after experimenting I found in some cases, after a few months that the cells of the canned fruits were actually losing sugar and that considerable carbon dioxid was being set free. After applying heat sufficient to kill the cell life of such fruit, the phenomenon is no longer observed. *Canned fruits will therefore undergo spontaneous decomposition* if sufficient heat is not employed in the sterilizing process to destroy the life of the cells. The fruit flavor suffers to some extent from the extended sterilization, but the trouble and loss is avoided. One other fact deserves mention in this connection and that is the temperature which develops spring bottoms. If the cans are stored in a temperature of 40° to 50° F. and opened before being allowed to reach a warmer temperature, even the underprocessed fruits will be found to be free from partial decomposition. It usually happens that the trouble is experienced after the cases are brought out for sale in the early summer, just before the fruit season opens. Wholesale grocers who buy heavily in the fall and store the goods, usually experience some trouble when the cases are brought out in warmer weather for the trade.

There is another cause of decomposition, and that is the influence of light on canned goods, particularly foods canned in glass. This is true of foods containing tartaric acid, glucose, lactose and maltose, etc., especially if the foods are faintly alkaline or if alkalies

are present even in small quantities. The action of sunlight on exposed solutions containing tartaric acid may be expressed by the following chemical equation:

$C_4H_6O_6 + 3O = 2CH_2O_2 + 2CO_2 + H_2O.$

Tartaric acid + Oxygen = Formic acid + Carbon dioxide + Water.

Glucose and lactose have been found on exposure to sunlight in hermetically sealed packages to break down and form alcohol and carbon dioxid, or just the same fermentation that is caused directly by the yeast plants (Saccharomyces). The same two substances may yield, in the presence of lime, lactic acid and carbon dioxid, or a fermentation corresponding to that produced by the bacteria which cause the souring of milk. Thus maltose is broken up, and yields dextro-lactic acid; levulose yields levo-lactic acid, and invert sugar will yield an inactive acid, when polarized.

A word of advice to canners of food products in glass might be opportune in this connection. Wrap your glass goods well, and in each case put cards requesting the retailer not to remove the paper when he places his goods on the shelf. All such goods should be neatly wrapped and have labels on the outside sufficiently attractive for the shelf. Goods unwrapped for show windows should be sold very soon on account of the danger of chemical changes noted above.

The word fermentation has a wide meaning, in fact it is a word to which various definitions have been given not in accord with its root meaning, and it is made to embrace all such transformations and decompositions as we have just described, so we return to the word micro-decomposition as one which defines the changes produced by fungi directly.

Bacteria molds and yeasts are nutrient food substances to build up cell protoplasm, and this is followed, or there goes on at the same time, an excretion of waste materials which have received the names of enzymes and toxins. There are formed at the same time various acids and chemical compounds as a result of the disturbances caused by the utilization, by bacteria, of certain elements such as carbon, oxygen, hydrogen and nitrogen, which are torn from the molecules containing them, thus setting free other atoms which unite to form those products of decomposition. To make this clear to the reader not familiar with chemistry it may be explained thus: A molecule is the smallest body conceivable which retains the identity of the substance, and this molecule is formed by two or more atoms or elements. An atom is an element. The atoms are united to one another in certain relations which form the different substances with which we are familiar; thus alcohol is expressed by its molecular symbol C_2H_6O, which means that two atoms of carbon,

six atoms of hydrogen and one atom of oxygen are united. Now if a fluid containing a limited quantity of this alcohol (less than 15 per cent.) is planted with the acetic acid bacteria in the presence of atmospheric oxygen, the germs will use from the alcohol two atoms of hydrogen and from the air two atoms of oxygen, and the result is that the alcohol is changed into acetic acid and water thus:

$C_2H_6O + O_2 = C_2H_4O_2 + H_2O.$

Alcohol + Oxygen = Acetic acid + Water.

Substances undergoing micro-decomposition usually contain various species of bacteria and the chemical compounds produced are often very complex for the reason that each species may be transforming the same substance into characteristic compounds, and the acids and compounds formed by one species may be attacked and changed by a different species into different compounds. For instance, the yeast plants may be producing alcohol by their action on glucose, and at the same time the acetic acid group will seize on the alcohol produced and convert it into acetic acid, and this acid may be attacked by still another species and converted into carbonic acid and water. In order to study with accuracy the compounds formed by a given species it is evident that pure cultures of that species must be obtained and grown in a favorable nutrient medium. The separation of pure cultures is comparatively easy by the methods established by Dr. Koch of growing them in solid media such as gelatin and agar, which confines the different species to isolated positions where they may be transplanted to other media in unmixed cultures.

There are usually several products resulting from the vital activity of a given species, thus the yeast plants produce alcohol, carbonic acid, succinic acid, glycerin and some volatile acids. There are many varieties of yeast plants which produce these products in varying quantities; some species yield very large amounts of alcohol and are specially cultivated for brewing, baking, etc. The products elaborated by them depend largely upon the material in which they are growing, and this is true of all bacteria as well. Vital activity goes on as long as fresh material is added until a certain per cent. of waste product is produced, when they cease to perform their functions; then they become dormant or actually die under the influence of the chemicals formed during vital activity. Thus the yeasts will multiply until about 15 per cent. of alcohol is produced. In addition to the products mentioned above the yeasts or saccharomyces produce an enzyme which is a soluble ferment capable of producing alcoholic fermentation after the germs are dead, if placed in fresh nutrient media. There are a number of molds which produce alcohol and various acids when submerged in fermentable materials. Oxygen is required in large quantities by

the molds and when this is cut off by excluding the atmosphere, they seize the oxygen which is in combination and a true fermentation, resembling that of yeasts, is produced. The free admission of atmospheric oxygen lessens the fermentation, since this requirement is more easily appropriated than that which is in chemical combination. The fermentation will be accomplished completely in a longer time, however, since the evolution of gas formed cuts off the supply of atmospheric oxygen and the chemical combinations are broken down for their supply. The vacuum therefore is a good condition for alcoholic fermentation. Multiplication is not so prolific but fermentation is more violent and considerable heat is generated, due to the breaking up of molecules and the formation of new chemical compounds. Alcoholic fermentation was formerly allowed to go on slowly for months in the breweries, but the process has been greatly shortened by the vacuum process. The vacuum pumps are set to work and the oxygen and gases are pumped away from the fluids, thus compelling the yeast to break up the sugar more rapidly, for their supply of oxygen.

A great many bacteria which grow naturally and luxuriantly in the presence of air, are thus enabled to cause more violent fermentation when air is excluded or when they are compelled to grow in vacuo. Thus we see that a vacuum has no value as a means of preventing fermentation. In canning fruits and vegetables in tin cans a vacuum is desirable, not for the prevention of fermentation but to cause the ends to draw in after the sterilizing process. During this process the ends of tin cans become bulged and unless a vacuum is present, after cooling they draw in very slowly or not at all. The vacuum is produced by heating the contents before finally sealing the cans or by mechanical means, the power depending upon the heat and fullness of the cans. The expansion of fluids is greatest at or near the boiling points and on cooling there is a corresponding contraction. When cans are not filled full and the contents are quite hot the vacuum formed on cooling has great power, often causing the cans to collapse. The vacuum may be regulated by attention to the heat and fullness of the can. A temperature of 180° F. and filling as full as possible will produce a vacuum of sufficient power for all practical purposes. The vacuum has value in the detection of swells; cans which do not draw in are likely to be either leaks or swells. In this connection I want to call attention to the misrepresentations of certain manufacturers of vacuum machines. Recently a circular reached me giving glowing accounts of a machine capable of sealing in vacuo thousands of cans daily, doing away entirely with the sterilizing process, and claiming great saving in steam and labor and the preservation of natural flavors. The whole process was described, which consisted

Saccharomyces Cerevisiae

Origin.—Beer or bakers' yeast; also found in the air.

Color.—White.

Form.—Cells spherical or egg-shaped 8-10μ broad; they are colorless, and have a homogeneous protoplasm when actively growing. Granules and vacuoles develop later. Zoogleal masses may be formed, owing to a gelatinous exudate. The cells are sometimes single, sometimes they have several buds; long, branching forms are found at times, especially above 30°.

Motility.—Is not motile.

Sporulation.—Several spores form usually. These may be double stained. They develop between 11° and 73° c.

Anilin Dyes.—Stain readily, as does also Gram's method.

Growth.—Thick white growth, which is particularly abundant on glucose media and in wort.

Gelatin Plates.—The colonies are small, white, opaque, circular in shape, very coarsely granular and slimy.

Stab Culture.—There is a thick white growth on the surface. No growth in lower portion.

Streak Culture.—On agar and on potato a thick, somewhat raised, white growth is formed.

Temperature.—Fermentation takes place most rapidly between 14° and 18° C., as an upper yeast.

Behavior to Gelatin. Does not liquefy.

Aerogenesis.—A ferment, invertin, is formed which changes cane-sugar into glucose. The latter is then changed to carbonic acid and alcohol (4-6%) by another ferment (zymase). Does not ferment lactose.

Pathogenesis.—No effect on animals. A catharrhal condition may be produced in the alimentary tract by a large amount.

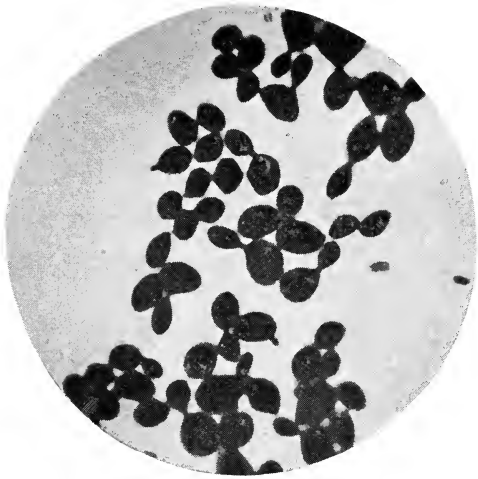
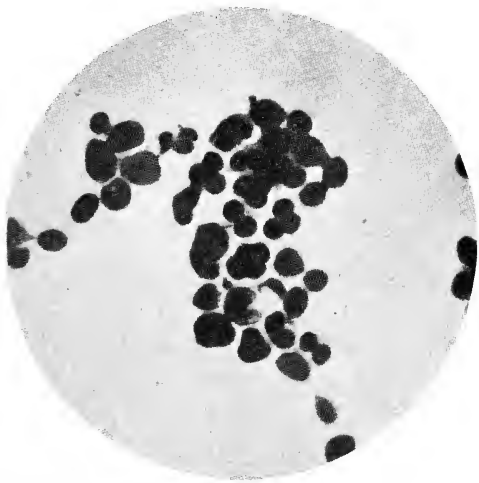


Plate 27

Saccharomyces cerevisiæ, showing budding cells. Potato culture. x 1000.



Saccharomyces cerevisiæ. Culture on plaster service. Stained with carbol-fuchsin and methylene-blue. x 1000.

of putting raw fruits, vegetables, meats, etc., in patent cans, which were run into the vacuum machine where the air was completely exhausted and the sealing was done in vacuo. I wrote these parties, requesting a detailed account of their process, for I supposed that there must be some sterilizing process connected with it, but to my astonishment they claimed that the vaccum produced was all that was necessary. There are a number of canners who look upon a vacuum as a necessary condition. The fact is, however, that a vacuum is one of the best conditions for decomposition when certain species of living bacteria and spores are present.

THE VACUUM THEORY.

There seems to be such a widespread misconception of the true value of a vacuum in canned goods that a careful study of the theory may not be out of place at this time. Occasionally new machines are advertised for packing all sorts of goods by the "vacuum method," the advertisement reading that goods are superior in flavor and require less cooking, perhaps none, if this or that method is employed. In years gone by, nearly every packer believed that a vacuum in his cans was absolutely necessary for perfect keeping of the goods. The method generally adopted for obtaining the vacuum was to heat the cans in boiling water with vent holes open; the cans were then taken out and the holes were soldered or "tipped," after which the cans were ready for sterilization or the final process. Another method was to seal the cans completely; then they were given five or ten minutes boiling, after which each can was punctured with an awl, thus permitting the steam and gases (if any were present) to escape; then the awl holes were quickly closed prior to the sterilizing process. This method was called venting. The object of these two methods was two-fold, viz., to drive off any gases present and to expand the contents by heat, so that a vacuum would form by contraction after cooling. Another method which is used largely today, is to heat the goods before filling, then the cans are sealed while hot, and when they are cooled off after sterilization a vacuum is necessarily produced by the physical law of contraction.

It is very convenient and necessary that a vacuum be formed in tin cans so that the ends will draw in after the sterilizing process. It would be impossible to drive the ends back in some cases (depending, of course, upon the goods) unless this vacuum were formed. There is one exception, that is, cold packed tomatoes; but this cannot be called a true exception, because the tomatoes are generally warmer during the canning than they are after the cans have been passed through the final process and allowed to cool. Even then it is necessary at times to force the ends back to their

natural position; this is called "snapping." It is generally thought by packers that all cans are sound which give evidence of a vacuum, and this idea has given rise to the belief that a vacuum actually keeps the goods from spoiling. It is indeed surprising how generally this error has crept into the minds of packers and certain manufacturers of vacuum machinery, too. Only two years ago a certain manufacturer declared to me that he was able to reduce the time of sterilization by twenty to twenty-five per cent. He claimed to have the records to prove that his assertions were correct. Another manufacturer distributed broadcast a circular describing his new vacuum machinery, by means of which he claimed to be able to can fresh fruits and vegetables without heating (as far as I was able to learn), and these he claimed would keep indefinitely without fermenting or decomposing. This machine was manufactured in Chicago, and its maker claimed that two canning houses were running 40,000 cans daily by this method. I wrote to him and warned him of the results which must surely follow, and told him if I did not comprehend his system fully, I would be pleased to be corrected. His reply contained the same claims, but I have never heard of the two canneries who were putting out 40,000 cans daily with his machine.

There are some machines on the market which have some merit as vacuum machines, because they exhaust the air from the cans while the contents are cold. This system is particularly attractive for some goods, such as meats; it accomplishes the same purpose as the old venting method, and the cold cans are more easily handled. By the old method it was necessary to heat the cans through to the center, which required a prolonged venting process. Every canner of meats remembers the time when the whole place was smeared with the grease which squirted out from the awl holes necessary in venting. The modern vacuum machine entirely does away with all that extra labor, inconvenience and unsightliness. This vacuum machine is made with a circular chamber, into which a dozen or more cans are carried around a sprocket wheel. When the machine is filled the chamber is closed and the air is exhausted by a vacuum pump. Each can has been previously capped, but the vent hole is left open, and the air is exhausted from the cans through the vent holes. Near the vent hole is placed a small button of solder with the necessary flux, and as the cans revolve they pass under a window and are tipped in vacuo by means of a tipping iron heated by electricity. The iron is not automatic but is controlled by the operator from the outside. A small electric light inside the chamber furnishes the illumination. As each can is brought under the window the small piece of solder is melted over the vent hole.

When the cans are all tipped, the vacuum is released, and the cans are carried out of the machine and carefully inspected for leaks.

As we have stated, the value of this device is the saving of time and the neatness of the work. It does not decrease the time required for sterilization, but does save the expense of venting.

The vacuum has no advantage as a means of shortening the sterilization of any canned goods. To understand this thoroughly let us study the character of the bacteria which are responsible for the spoilage of canned goods. There are a great number of bacteria yeasts and molds which will cause chemical changes in canned goods unless they are destroyed by sterilization.

Nearly all the bacteria which cause the spoilage of canned goods after incomplete sterilization are spore-bearing organisms. If there should happen to be a leak in the can, or should processing be neglected, non-sporating varieties would set up decomposition. Non-sporulating varieties are always destroyed at boiling temperature (212° F.) All spore-bearing bacteria which are responsible for spoilage in canned goods are either anaerobic or facultative anaerobic; that is to say; some are able to grow only when oxygen is entirely absent, and some are able to adapt themselves to either condition. The vacuum, then, is an ideal condition for the growth of anaerobic bacteria, because the stronger the vacuum, the better the environment. The least trace of oxygen interferes greatly with the multiplication of these germs. When we speak of oxygen in this connection, we mean free oxygen as it is found in the atmosphere. The anaerobic bacteria do require oxygen, but not in the free state; their supply is always obtained from molecules of nutrient substances which have oxygen chemically combined with other atoms. In chemistry we speak of any substance as being made up of molecules, and the molecules as being made up of atoms chemically combined. A molecule is defined as a very small particle of matter which has all the characteristics of the natural substance; for instance, a molecule of sugar is the smallest particle which has all the characteristics of sugar. A molecule cannot be farther divided without destroying its character. Thus we may illustrate $C_6H_{12}O_6$ is a molecule of grape sugar which is fermented by the lactic acid bacteria will be divided thus:

$$\left.\begin{matrix} C_6H_{12}O_6 \\ \text{Grape Sugar} \end{matrix}\right\} = 2 \left\{\begin{matrix} C_3H_6O_3 \\ \text{Lactic Acid} \end{matrix}\right.$$

which would read thus: One molecule of grape sugar is divided into two molecules of Lactic Acid. A molecule is composed of natural elements called atoms, and each atom is designated by a letter, thus $C_6H_{12}O_6$ means that a molecule of grape sugar is composed of 6 atoms of Carbon, 12 atoms of Hydrogen and 6 atoms of Oxygen, and when this combination is broken up other sub-

stances are formed. As we have shown, the molecule of grape sugar is changed by lactic fermentation into two molecules of Lactic Acid. Now if we let grape sugar ferment under the influence of yeast or mold the following takes place:

$$\left.\begin{matrix} C_6H_{12}O_6 \\ \text{1 mol. grape sugar} \end{matrix}\right\} = \left\{\begin{matrix} 2\ C_2H_6O \\ \text{2 mols. alcohol} \end{matrix}\right\} + \left\{\begin{matrix} 2\ CO_2 \\ \text{2 mols. of carbon dioxid} \end{matrix}\right.$$

Then again if we let grape sugar ferment under the influence of the Acetic Acid bacteria we have the following:

$$\left.\begin{matrix} C_6H_{12}O_6 \\ \text{1 mol. grape sugar} \end{matrix}\right\} = \left\{\begin{matrix} 3\ C_2H_4O_2 \\ \text{3 mols. of acetic acid} \end{matrix}\right.$$

These chemical equations illustrate the fact that a molecule is entirely changed in character when it is divided. This gives a splendid idea of chemical changes brought about by different organisms, although in reality they are still more complicated, so that instead of Alcohol, Lactic or Acetic Acid being formed alone, there are usually several other complex substances formed at the same time, such as glycerin, succinic acid and volatile fatty acids.

Plate 28. Aspergillus Glaucus

Aspergillus Glaucus, showing the conidia on the tufts or sporangia. Magnified 350 diameters

Our readers will notice that in the fermentation of grape sugar, the different atoms are torn apart, and the particular organism responsible for the fermentation uses the elements for its propaga-

tion. Nothing is entirely lost chemically and although carbon, hydrogen and oxygen are used to build up cell protoplasm, those elements unite promptly to form the products elaborated by the germs, and of course are characteristic of them.

The Anaerobic bacteria, therefore, obtain their supply of oxygen from chemical combinations. This is true also of other bacteria which are forced to grow in an anaerobic condition. The process of decomposition is therefore more complete where the air is entirely excluded from such micro-organisms, and the vacuum in the cans is a favorable environment. The molds, yeasts or bacteria, which consume large quantities of oxygen, must obtain that element, consequently a much larger quantity of material must be changed quickly for the supply of oxygen. In such cases the number of germs present is quite small in comparison to the amount of ma-

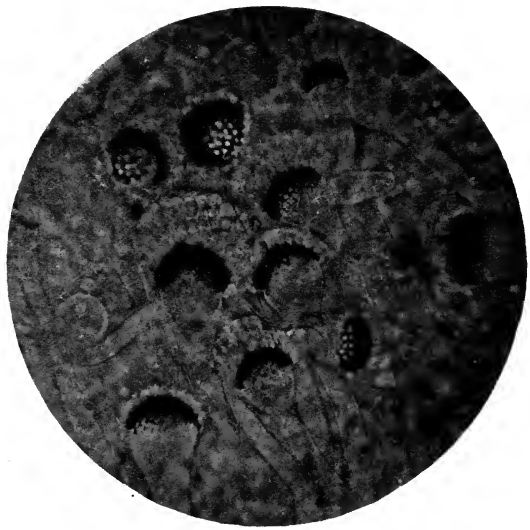


Plate 29. Aspergillus Glaucus

Photomicrograph of the beautiful mold plant Aspergillus Glaucus. The fruit hyphae showing the bottle-shaped sterigmae radiating from the columellae and the conidia are plainly visible. This is an unstained specimen mounted in glycerine and photographed. Magnified 500 diameters.

terial which is undergoing chemical change. Oxygen is more difficult for bacteria to obtain when they are forced to grow in a vacuum, because large quantities of material must be deprived of oxygen. Decomposition is generally pretty well advanced when spoilage is noticed in canned goods, because the vacuum of the can has deprived the bacteria of free oxygen. Sterilization must therefore be complete, if goods are to be kept pure and unfermented in tin cans or glass. The vacuum has absolutely nothing to do with the keeping quality of the goods, and we might add that the vacuum

is a favorable condition for decomposition, unless sterilization is complete.

Test tubes containing beef juice, corn juice, peas, etc., are easily sterilized with only a cotton plug in the top; there is no vacuum, but the germs from the air are filtered out by the cotton. This is the method used in the laboratory for sterilizing culture media.

Any canner may test the value of a vacuum for himself as follows: Take a can of any perfectly sterilized goods, heat an awl in a flame until it is red, heat a small surface of the can, holding flame directly onto it; then punch a hole in the can without removing flame, then seal the hole. The vacuum will suck air into the can through the awl-hole but the air must pass through the flame which destroys all molds and bacteria. Although the vacuum has been destroyed by the admission of heated air, the contents of the can will remain in an unfermented condition.

CONCLUSIONS.

Molds, yeasts, anaerobic bacteria and bacteria which are facultatively anaerobic, will grow in a vacuum, on a nutrient medium. There is no such condition as an absolute vacuum in nature, but there may be a condition where there is a partial vacuum, where atmospheric oxygen is entirely absent or replaced by some other gas. A vacuum will not prevent decomposition. Decomposition by bacteria is more complete when air is entirely excluded from canned goods. Sterilization cannot be accomplished in any less time in the presence of a vacuum, since it requires a certain amount of heat, which must be applied for a given time, to destroy spores of bacteria, yeasts and molds.

VALUE OF VACUUM MACHINERY.

Vacuum machinery may have some advantage over other methods of obtaining an exhaustion of air, viz., goods may be handled cold and considerable labor of venting is saved. For reducing the bulk of any goods such as milk where a high temperature is liable to injure the flavor, a vacuum is valuable for removing the atmospheric pressure, so that ebullition may take place at a comparatively low temperature. Evaporation by this method has no value as a sterilizing process where spore-bearing bacteria are present; it requires the high temperature to destroy spores and the vacuum system cannot give successful results in any less time than is actually required where that condition is absent. Goods like milk, which are condensed by boiling in vacuo, are not sterilized, but are preserved by sugar which must be added up to 50 per cent. in some

cases. Sugar is a preservative when used in large quantities, because it takes up the fluids. Bacteria require fluids for multiplication, so when sugar is used in excess the spores are deprived of fluid, and therefore remain dormant, but are not destroyed.

Before closing, let me remark that no vacuum pump is able to remove all bacteria from any goods. It may remove a large number from the small air space at the top of a package, but it cannot exclude those forms which are in the goods themselves.

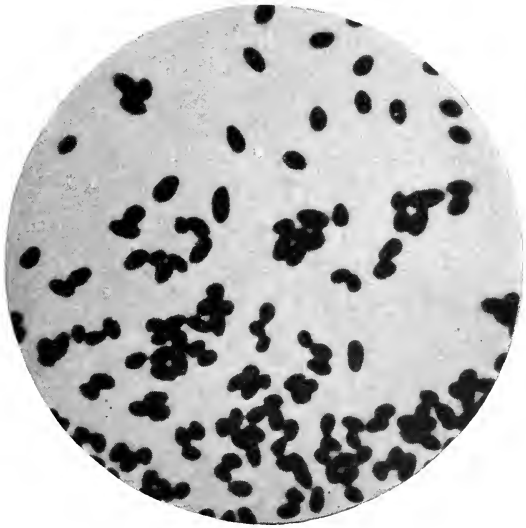


Plate 30. Saccharomyces Ellipsoideus

Photomicrograph of a wild yeast or wine ferment Saccharomyces-Ellipsoideus (Hansen) round or oval cells, which produce spores 2 to 4 *microns* in diameter, two or four being found in a single ascus. It forms a delicate surface film in about two weeks at 75 degrees Fahrenheit. It produces a rapid and powerful fermentation, with formation of great quantities of carbonic acid gas. Magnified 800 diameters.

The yeasts and molds are not the only species which produce alcoholic fermentation; there are a number of bacteria which convert glycerin media into alcohol; the typhoid and pneumonia bacilli, also a number of bacteria found in the mouth and on the teeth have the same power. Some of the mucors are employed in pure cultures in the manufacture of alcohol.

The yeasts cannot convert starch into alcohol, so in some places molds are employed to convert the starch into sugar and the yeast is then introduced to convert the sugar into alcohol. In the manufacture of malt beverages and vinegar the diastase is first employed to convert the starch into sugar and this in turn is converted into alcohol by yeast. Maltose and cane sugar are changed by a ferment produced by yeasts, into glucose, which in turn is converted into alcohol and the by-products. The chemical formulas are as follows:

$$\left.\begin{matrix} C_{12}H_{22}O_{11} + H_2O \\ \text{Cane sugar} + \text{water} \end{matrix}\right\} = \left\{\begin{matrix} C_6H_{12}O_6 + C_6H_{12}O_6 \\ \text{Dextrose} + \text{Levulose} \end{matrix}\right.$$

$$\left.\begin{matrix} C_6H_{12}O_6 \\ \text{Invert sugar} \end{matrix}\right\} = \left\{\begin{matrix} {}_2C_2H_6O + 2CO_2 \\ \text{Alcohol} + \text{Carbonic acid} \end{matrix}\right.$$

The use of glucose in jellies, jams, catsup and other food products is therefore fraught with danger. The atmosphere is laden with wild yeasts which easily attack the glucose and fermentation quickly follows. It has been customary among jelly and preserve makers to adulterate the juices with glucose to give body and to sweeten with saccharin, using some antispetic to retard fermentation. It is better to produce pure goods, although it may be advisable in some cases to use a preservative, but the label should plainly state the fact. Large quantities of glucose are mixed with syrups and molasses to produce mild syrups, the object being to produce a syrup of milder and more delicate flavor and not to adulterate. These syrups are hard to keep and should be sealed hermetically and sterilized and not preserved with antiseptics. The manufacturers of syrups have a great deal of trouble along this line.

ALCOHOLIC FERMENTATION is probably the most useful chemical change accomplished by the lower vegetable orders. By this process all alcoholic beverages and commercial alcohol are manufactured. This fermentation is accomplished without the unpleasant odors and flavors so characteristic of many species of bacteria. The baking industry employs the yeasts and bacteria of alcoholic fermentation to produce the carbonic acid gas for raising or inflating what would otherwise be a heavy mass of dough.

In the preparation of sauces, catsups, syrups, jams, preserves, jellies and food products of like nature the first fermentation is generally alcoholic, due more commonly to the mold fungi but often to wild yeasts so abundant in the atmosphere. The seed forms of molds (called conida) give rise to this fermentation.

The conida are small round spores which abound on the tufts of many varieties of mold and when they are submerged will bud and multiply similar in many respects to the true yeasts or saccharomyces. Mold naturally grows on the surface of media which have a slight acid reaction, and its oxygen requirement is very great. So long as free oxygen of the atmosphere is to be obtained it grows luxuriantly without causing any fermentation of the lower parts of the material on which it is found, but if the oxygen is cut off either by submerging or enclosing, it is forced to obtain its oxygen requirement from the molecules in which oxygen is combined, and new compounds are thus formed and a fermentation is set up which in many respects resembles that of the yeasts.

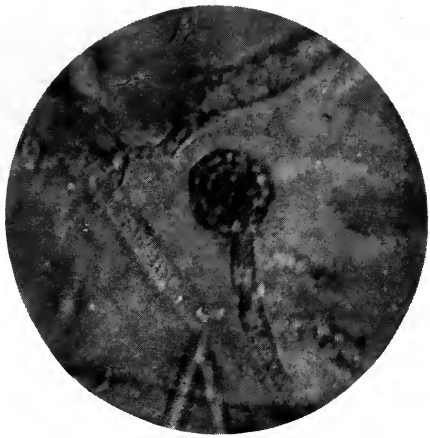


Plate 31. Mucor Mucedo

Photomicrograph of Mucor Mucedo in the living state mounted in glycerine. The round pod in the center contains the seed forms or conidia. This pod is ripe, ready to burst when the conidia are carried by water or air, ready to start a new mold plant or to set up fermentation according to the conditions in which they are thrown. Magnified 800 diameters.

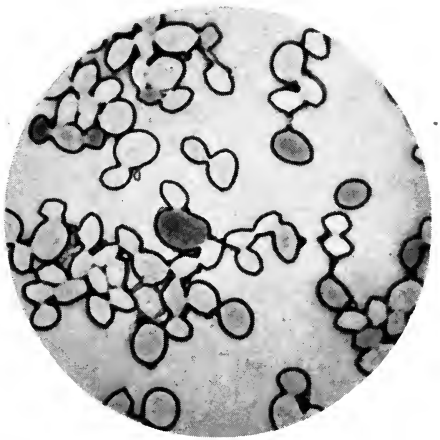


Plate 32. Mucor Mucedo, showing budding conidia

Photomicrograph of the budding conidia of Mucor Mucedo, obtained from a jar of spoiled tomatoes undergoing fermentation. These conidia have the power of setting up a fermentation similar in many respects to that of the yeasts. In this manner of growth Mucor Mucedo looks very much like the brewers' yeast Sacharomoyces Cerevisiæ. Magnified 1,000 diameters.

Various pulps are often filled into barrels hot, with a small amount of antiseptic to prevent fermentation, but on cooling quite a large air space is left above the surface of the pulp which is a rich field for the growth of mold. After the formation of mold the pulp will ferment if the barrel is rolled over and permitted to stand for a short time in any temperature above 34° F. This accounts for the large losses of manufacturers, who load and ship cars of barreled pulp from one place to another during the spring of the year. The pulp when stored away in cellars remains quiet for months and appears good, but too frequently the mold is present, and loss follows the moving. This may be overcome to some extent by boiling pulp down to 25 per cent. solids and refilling barrels after cooling. Pulp stored in barrels will not keep unless a small amount of preservative is added. The loss from alcoholic fermentation may be minimized by canning the pulp in large tin cans and processing. This insures a far better quality and does away with the necessity of using antiseptics.

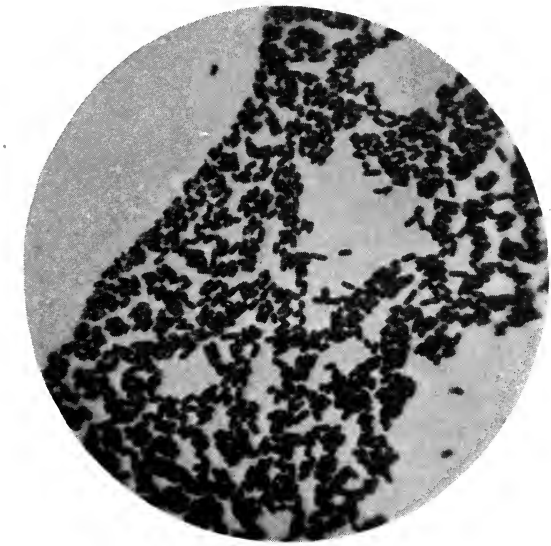


Plate 33. Acetic Acid Bacteria

Photomicrograph of the vinegar bacillus, Bacillus Acidi Aceti, which was isolated from a leaky can of tomatoes. This is one of the organisms which are usually found in the "mother" of vinegar, which is called Mycoderm Aceti. Solutions containing alcohol in amounts less than 15 per cent. are fermented and the alcohol is converted into acetic acid. Stained with fuchsine and photographed through the microscope. Magnified 1,200 diameters.

ACETIC ACID FERMENTATION.

Acetic acid fermentation is one of the most important chemical changes produced by bacteria. Vinegar is the chief commerical product obtained, and while as yet pure cultures of bacteria

are not extensively employed to produce it, there is a possibility that present methods may be superseded and a vinegar of greater strength obtained by the utilization of a single species known to have the power to produce a higher per cent. of acetic acid than when grown in company with other organisms.

There are a number of bacteria which produce acetic acid when grown in liquids containing not more than 15 per cent. alcohol. Pasteur demonstrated that acetic fermentation was due to the living organisms which formed slimy scum on the surface of alcoholic fluids such as beer, cider, wine, etc. This scum was named "Mycoderma aceti," which means "germ skin of acetic acid" or "mother of vinegar." It is a zooglea mass of various bacteria, such as Bacterium aceti, B. Pasteurianus, Bacillus aceticus, Bacterium Kutzingianum, etc.

Acetic fermentation is common and develops rapidly in the alcoholic fluids when exposed to the atmosphere at a temperature of 80° to 85° F., which is most favorable. Under favorable conditions with pure cultures 14 per cent. acetic acid may be formed, which is equal to 140 grain vinegar. The temperature given above must be lowered after the acetic germs have performed their functions to prevent oxidation of the acetic acid and water by the bacteria still living in the mycoderma. The chemical equations for these two changes are symbolized as follows:

1 $= C_2H_6O + O_2 = C_2H_4O_2 + H_2O$

Alcohol + oxygen = Acetic acid + water.

2 $= C_2H_4O_2 + 2O_2 + 2\,CO_2 + 2\,H_2O$

Acetic acid + Oxygen = Carbonic acid + water.

In the "mother of vinegar" the germs are united very closely by mucinous envelopes, which are capsule-like formations, in which the bacteria are imbedded. Iodin stains these envelopes in a peculiar manner. Those of B. Pasteurianus and B. Kutsingianum are stained blue, while the germs themselves are not. The envelope of B. Aceti does not take the stain.

At the temperatures given, 80° to 85° F., involution forms occur.

By involution forms we mean that the bacteria change from their natural forms, as shown in Fig. 30 and form threads which are very much smaller in places and have no resemblance to the normal shapes.

As mentioned before, vinegar is the chief commerical product of these germs, and vinegar is formed from liquors containing not more than 15 per cent. alcohol. Oxygen is absolutely necessary for these organisms and they consume large quantities, and for this reason the vinegar spirit is diluted with vinegar and allowed to run slowly over beechwood shavings in a vinegar generator, thus ex-

posing the large surface to the atmosphere. These beechwood shavings are sown with acetic acid bacteria and a rapid acidification follows. By this exposure large quantities of alcohol are lost and there are many forms of bacteria which find their way into the spirit along with the acetic acid germs. Parasites such as Anguillula aceti, the so-called vinegar eels, and Pythium anguillulae aceti, make their appearance and consume both alcohol and acetic acid. There are many people who have the opinion that these parasites produce acetic acid, but such is not the case. The first named species is the more common, but the second belongs to the fungi group of

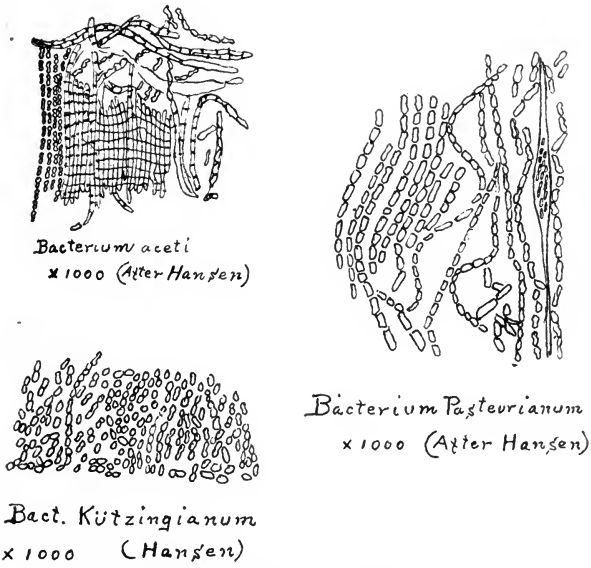


Fig. 30. Bacteria found in the Mycoderma Aceti

Oomycetes and these destroy the first species, as was discovered by Sadebeck. The utilization of pure cultures of acetic acid bacteria has not been accomplished outside the laboratory, but there is no doubt that it is possible and practical. A vinegar of 14 per cent. acetic acid strength may be manufactured with pure cultures from the very spirit which is yielding only nine, ten and eleven per cent. for the manufacturers under present conditions.

The additional yield of acetic acid means a great saving and is worth the attention of manufacturers. Pure cultures may be obtained easily by the plate culture methods, which were described in Chapter III. The spirit can be sterilized and sown with the pure culture and pure oxygen can be generated and forced into specially prepared tanks in such a manner as to exclude all foreign bacteria and parasites. Vinegar thus prepared will be of great strength and promises large returns for the successful apparatus.

The old method known as the Orleans method of manufacturing vinegar is still used in many places. The method is thus described by Lafar, page 397. "A number of oaken casks, each of a capacity of some 55 gallons, are arranged in rows in a chamber maintained at a constant temperature of 64° to 71° F. In the upper part of the head of each cask a circular aperture is provided, through which the cask is filled and emptied and which is generally kept closed whilst near it is a very small vent always left open for the admission of air. In normal work each cask is about half full. Before setting a new cask in work, it is scalded out several times with steam or hot water, in order to extract the sap from the wood, and is then 'soured' by impregnating it with good, boiling-hot vinegar. About 22 gallons of good, clear vinegar on less than ½ gallon of wine are then placed in the cask, another ½ gallon of wine being added at the end of eight days, more after the lapse of another week and so on until the cask contains 40 to 44 gallons." Vinegar is then drawn from the cask after the "mycoderma" has formed and this is replaced by the addition of wine. The cask is used for several years when deposits are so heavy as to necessitate emptying and cleaning."

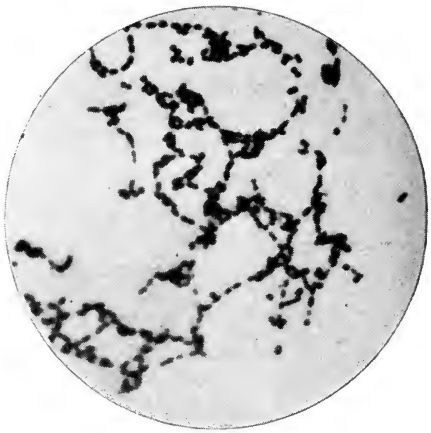


Plate 34. Acetic Acid Bacteria

Photomicrograph of Bacillus Acidi Aceti or Mycoderma Aceti or "Mother of Vinegar," showing short dumb-bell rods, large lemon shaped and drumstick, involution forms. Produced acetic acid in tomatoes, isolated by plate culture method; stained with fuchsine and mounted in xylol balsam. Magnified 1,000 diameters.

The slowness of this method is apparent and the opportunity for contamination by injurious bacteria is great. There are enormous losses of alcohol and acetic acid and the quality of the vinegar is often very poor. This process was improved (?) by Pasteur in 1862, who cultivated the "mycoderma" or "vinegar flowers" in small vessels and transferred this to the surface of the wine in vats kept open and exposed to the air for the supply of oxygen, but

the process produces various results due to contaminations by harmful bacteria. Pasteur's idea of cultivating the true acetic acid bacteria was good, but the apparatus is faulty. For this reason his methods are not now in favor, the quick vinegar method having taken its place to a very large extent.

These two processes have been outlined in this connection merely to point out the imperfections in them and not to describe the best method of manufacturing vinegar. By the present methods it is plain that the mixed germs employed in acetic fermentation do not accomplish the best reslts and that there is considerable loss in alcohol and acetic acid.

In general, acetic fermentation causes very little trouble as a source of spoilage in the food products industry. Wines used in table sauces and soups may suffer from it if left exposed to the atmosphere and the same is true of any product in which alcohol is present not to exceed 15 per cent. Pulps which have undergone alcoholic fermentation either on account of wild yeasts or molds will also undergo acetic fermentation along with other fermentations such as lactic and butyric. Manufacturers of tomato catsup who use barrel pulp can call to mind numerous instances where the pulp had turned into vinegar and other complex acids. The preservers have some difficulties, too; preserves, apple butter, peach butter and light syrup goods are subject to slight alcoholic fermentation, unless properly handled and sterilized, then acetic fermentation follows with the loss of sugar.

Dill pickles, pearl onions are salted with just enough salt to plasmolyze the harmful organisms, and alcohol is generated first, then follows acetic and lactic fermentations to produce vinegar having a characteristic flavor.

All vinegar having a certain per cent. of solids in liable to deterioration through the agency of harmful bacteria, hence storage in cool places is recommended for such as malt and cider vinegars.

BUTYRIC FERMENTATION.

There is a recipe for butyric fermentation given in some chemistries, as follows: Put into a 10 per cent. sugar solution a small quantity of chalk and cheese and keep this at a temperature of 77° to 86° F. The first fermentation that starts is lactic in which lactic acid and calcium lactate are produced; the next is the butyric fermentation which is set up by an anaerobic organism 2μ broad and from $2—15\mu$ long, which was discovered by Pasteur in 1861. Pasteur did not class this germ as belonging to the bacteria but considered it an animalcule, because it had a rapid movement. Its manner of vegetating, however, is now settled and it can positively

be said that it belongs to the fission fungi, because it multiplies by lengthening and dividing and forms spores. It is endowed with numerous flagella, growing out all over the surface of the cell and by means of these its rapid motion is attained.

There are a number of bacteria capable of producing butyric acid, some of which are anaerobic, while other are aerobic.

Prazmowski studied the cause of this fermentation and describes an organism which corresponds with the "vibrion butyrique" discovered by Pasteur and named it Clostridium butyricum. Another germ similar in many respects was named Butyricus amylobacter, which is so named because the cell contents resemble starch which turns blue with iodin staining. So closely are the two germs allied, however, that I believe them to belong to the same family. In 1884 Hueppe discovered a bacillus which grows in the presence of oxygen which he named Bacillus butyricus and another similar to this was later discovered in old cheese and named Clostridium foetidum, while from milk Bacillus liodermos has been obtained.

In butyric fermentation various compounds are produced such as butyl alcohol, butyric, acetic and carbonic acids, hydrogen and sulphuretted hydrogen, etc. The fats and carbohydrates are subject to this fermentation and a chemical equation may be thus symbolized to show the decomposition of glucose into butyric acid, carbonic acid and hydrogen.

$$\left.\begin{matrix} C_6H_{12}O_6 \\ \text{Glucose.} \end{matrix}\right\} = \left\{\begin{matrix} C_4H_8O_2 + 2CO_2 + 2H_2 \\ \text{Butyric acid} + \text{Carbonic acid} + \text{Hydrogen.} \end{matrix}\right.$$

There are two kinds of butyric acid, differentiated in organic chemistry as fermentation butyric and isomeric acid or isopropyl formic acid, which is not obtained by fermentation; both have the same chemical symbols but are differently arranged in atomical relation. Butyric acid fermentation can be observed and studied by our readers by boiling a small quantity of milk in a test tube and allowing lactic fermentation to take place, which precipitates calcium lactate, which is attacked by the butyric acid bacteria, and from this cultures may be obtained by the plate method.

Butyric bacteria, whether they belong to the aerobic or the anaerobic species form spores which are resistant to high temperatures. They are found on the leaves and fibre of nearly all kinds of vegetables and cereals ready to set up butyric decomposition whenever the conditions are favorable for their development. The cellulose or fibre is the part usually decomposed by these organisms. It is a remarkable fact that even paper, made from wood pulp, will dissolve in a fluid undergoing butyric fermentation. It must not be supposed that simple butyric acid is the only product elaborated by species of this group since there have been isolated certain organisms which produce sweet-smelling ethers and aromatic sub-

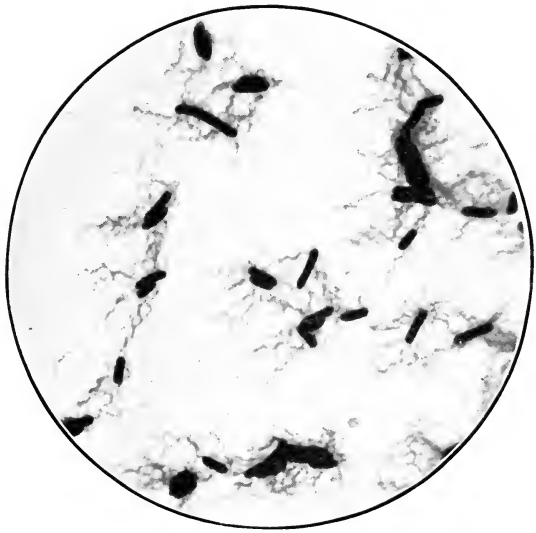


Plate 35. Bacillus Butyricus Amylobacter, Flagellated

Photomicrograph of Bacillus Butyricus Amylobacter, an aerobic bacillus which when grown on substances containing starch will stain blue with iodine. The flagella are very curly and were demonstrated by our special method, from a 24 hours' growth on 2 per cent. glucose agar which had been inoculated from the juice of corn in a swelled can. This organism is frequently found in decomposing vegetables and organic matter, and is not found in the air. Its habitat is probably the soil. Magnified 1,200 diameters.

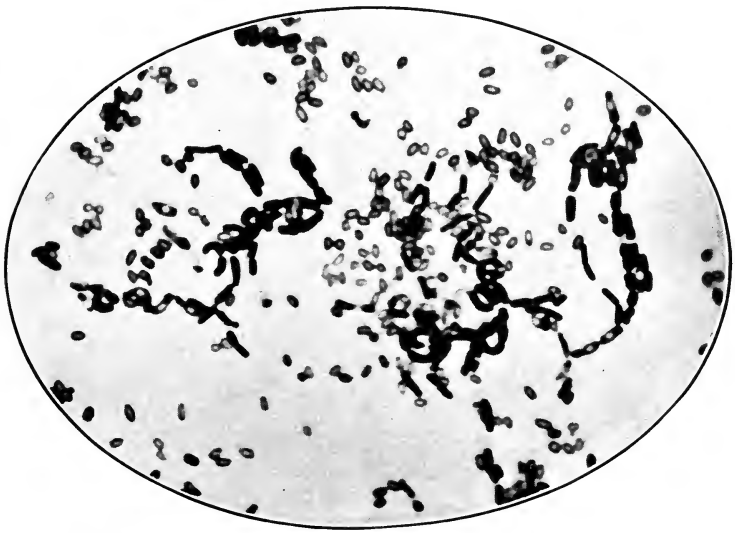


Plate 36. Bacillus Butyricus Amylobacter. Rods and Spores

This beautiful photomicrograph shows the free spores and the rods containing spores of Bacillus Butyricus Amylobacter. The spores are generally formed in the center of the rods which cause them to swell in the middle like spindles, hence they belong to the type called "clostridium." The spores are not easily destroyed by heat and may live in corn which has received a temperature of 250 degrees for nearly one hour. Stained with carbol fuchsine. Magnified 1,500 diameters.

stances which are valuable in the ripening of certain kinds of cheese, Among these might be mentioned Butyricus Amylobacter which we considered in the early part of this chapter, and Clostridum foetidumelactis, which gives the flavor to Limburg cheese, also a sugar-loving species found in soft country cheese called bacillus saccharobutyricus. Milk generally contains the spores of the butyric acid group, and it is due to them that the disagreeable odors which are associated with its decomposition are set free.

We make mention of cheese and milk and the bacteria associated with them because they enter largely into the formulas of special food products such as soups, macaroni and cheese (salad dressings), etc., sold on the market under private names. There are several different kinds of gases evolved where butyric fermentation is going on, H_2S (sulphuretted hydrogen), CO_2 (carbonic acid gas), CH_4 (methane or marsh gas). There are, however, some species which do not produce any gas. I isolated one species from cream of tomato soup, which had coverted the milk sugar and invert sugar directly into butyric acid without swelling the can. This species corresponded to the one isolated from soft cheese by V. Von Klecki, which he named bacillus saccharobutyricus mentioned above. The soup had been given a process of 250° F. for twenty-five minutes; the spoilage did not develop until a lapse of three weeks. It was found by experiment that one hour at 250° F. was necessary to destroy the spores of this organism. In some of the cans the regular well-known species were found, but the cans were swelled.

Butyric decomposition, while inimical to the canner's products, nevertheless has its uses in nature, and in some industries. It plays an important part in the preparation of brown hay, sweet ensilage and sour fodder, and in the aging of manures. In the retting of flax and hemp, the cellular substances are dissolved so that the fibre can be obtained in the pure state.

Butyric decomposition is one of special interest to canners of peas, string beans, asparagus, celery, corn and similar vegetables, because the spores of different types of butyric bacilli are present on surface of the pods and fibres. The butyric spores are usually ellipsoidal in form and not quite so broad as the mother germ; they withstand dessication remarkably well and high temperatures are required to destroy them. There are some other kinds of spore bearing germs which are more resistant than these, but not many; 250° F. for a few minutes will destroy the spores, but in order to get this temperature at the center of the cans the nature of the contents must be studied. If the material is heavy and thick and contains much fibre, it will require a much longer time than when the contents of the cans are strictly fluid. The exhausted cans are

not absolutely without oxygen since the space within contains some air, consequently strickly aerobic forms may be able to set up decomposition. Usually, however, the decomposition is set up by anaerobes and facultative anaerobes. Since almost all butyric bacteria belong to these two classes the conditions in canned vegetables are specially favorable for their vital activity.

The canning of such vegetables as are liable to butyric decomposition should be done very soon after they are harvested. The growing plants are not favorable for the invasion of these bacteria and it is only after they are harvested and attacked by other germs such as the lactic acid group that the hardier forms begin their work of destruction. They are specially active on vegetables which are par-boiled and allowed to stand exposed and thus demonstrate that they are true scavengers.

The development of spores and the formation of spores is accomplished in from thirty to forty-five minutes by the butyric bacteria when every condition is favorable. When partly cooked vegetables are allowed to stand too long the center of the mass will be attacked by the anaerobic forms while the aerobic forms on the surface are thriving. Those on the surface use up the oxygen from the air, at the same time set free various gases and in this way create the most favorable conditions for the development of the anaerobic variety, the spores of which are scattered within the mass. This is also true of the raw material which is piled too closely. Here the peptonizing ferments begin to vegetate and soften the fibre, causing the juices to ooze through their protecting sacs and the temperature is increased so that the appearance of the vegetables resembles par-boiling; in other words, they look as if they were cooked. This is the condition as before described and butyric decomposition progresses rapidly. A bitter flavor is often imparted to such vegetables by a bacillus, which has given me considerable trouble to isolate. It is a spore-bearing bacillus actively motile, due to a large number of flagella. The spores are very hardy, requiring about fifteen minutes at 250° F. to destroy them. They are oval and located in the center of the rods which at times give the bacillus a clostridium appearance. The development of the spore is similar to that of the Bacillus megatherium of De Bary. The young rod passes out of the spore at right angles to the long axis of the spore and often retains the spore shell at one or both sides. The bacillus is from 2μ to 6μ long and about 0.8μ to 1μ broad, with rounded ends similar to B. subtilis. It forms butyric acid and coagulates milk, is a facultative anaerobe and does not cause the cans to swell. Cans of peas, asparagus and string beans inoculated with a pure culture turn quite bitter within six days. The colonies growing on agar are round with a pale, transparent,

very delicate zone surrounding them; the surface is white, slightly wrinkled, becoming more so with age. When magnified by 250 they are yellowish and opaque. The deep colonies have a whetstone appearance. Grows well at a temperature of 85° to 90° F.

As bitter decomposition has caused the canners considerable trouble at times the description here given will be interesting. The bacillus may produce the bitterness in the raw material if too long exposed or to the partially cooked products if allowed to stand too long before the final process, or it may develop in the cans if underprocessed. The spores are destroyed, however, at a temperature of 250° F. for fifteen to twenty minutes' actual heat; time required for penetration of can and the contents must be added to this.

Frequently I have noticed that goods which have undergone chemical changes due to bacteria have no living bacteria in the cans. When the fluid is examined under the microscope there are numerous bacteria present, but when agar or gelatin plates are inoculated there would be no growth. These bacteria when mounted fail to take the stains readily, which proves that they are dead. This phenomenon will be noticed frequently by our students in their researches and is explained in two ways; either the bacteria were destroyed during the sterilizing process, having previously accomplished the chemical changes, or they died under the influence of the products elaborated by themselves, either before the sterilizing process or more likely afterwards. If vital activity goes on after the sterilizing process, the amount of acid produced is often germicidal, and this may be accomplished in a few days; it is usually after a longer time, however, varying from three weeks to six months. The examination of spoiled cans should be made as soon as possible after the trouble develops.

A great deal of the trouble experienced from butyric decomposition is avoided by careful attention to cleanliness. Remember that the floors and machinery will be covered more or less with accumulations each day and this dirt contains the elements of what you are packing. Failure to remove such accumulations by the liberal use of soap and water, and at times a powerful disinfectant, only invites an inimical host of bacteria, which is ready to attack the fresh product which is being canned. The waste material should be moved far away from the factory, and where practical it may be put into silos and converted into money where a market is offered for ensilage. The habit many packers have of loading wheelbarrows and dumping cobs, husks, pea-pods, peelings and waste just outside of the factory in great piles, is very dangerous. While you may have been successful in one season with a certain time and temperature in your sterilizing process, you may have great difficulty during the next season. The atmosphere in the

Bacillus Megatherium, De Bary

Origin.—Found originally on boiled cabbage leaves; is present in the air and in the soil; it is also found on other vegetable matter.

Form.—Large cylindrical rods, having rounded ends, three to six times as long as broad, and with granular contents. They are found in pairs, ordinarily slightly bent; they may form threads. Involution forms are quite common, and capsulated cells are especially found in slimy growths.

Motility.—They have six to eight flagella, and have a slow, creeping motion.

Sporulation.—Median spores are formed.

Anilin Dyes.—Stain readily, though irregularities may be seen which are due to granular protoplasm.

Growth.—Rapid.

Gelatin Plates.—Small, irregular, yellowish colonies are formed, which later show marked branching or radiating forms—these soon liquefy the gelatin. Sometimes the colonies are kidney-shaped.

Stab Cultures.—The growth is rapid, attended with liquefaction along the line of inoculation, and may show threads of bacteria penetrating outward into the solid gelatin. The gelatin becomes wholly liquefied later, a flocculent mass accumulating on the bottom; the liquid clears up without any formation of scum on top.

Streak Cultures.—On agar, a dull white or grayish covering is formed. On potato, a thick, slimy, grayish-white mass is rapidly formed, rich in spores and involution forms.

Oxygen Requirements.—Aerobic.

Temperature.—May grow in incubator, but optimum heat is at about 20° C.

Behavior to Gelatin.—Liquefies rather slowly.

Pathogenesis.—No effect has been observed.

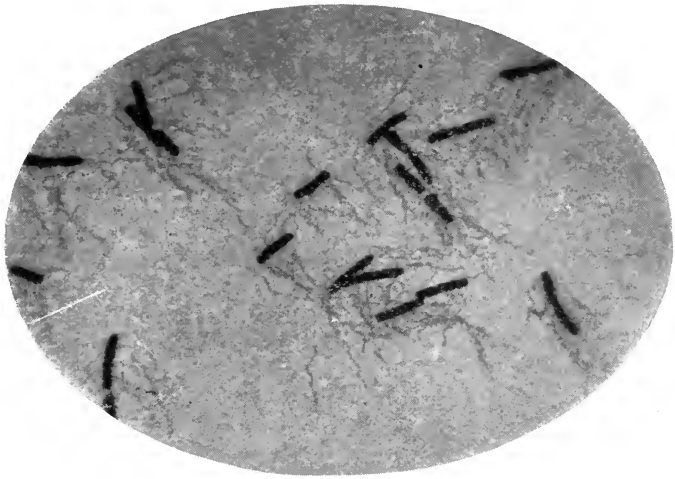


Plate 37. Bacillus Megatherium, Flagellated

Photomicrograph of an actively motile, spore-bearing bacillus which belongs to the Magatherium group. It was isolated from some pickles to which it had imparted a most disagreeably acrid, bitter flavor. It requires an acid medium for luxuriant growth, diluted malt and cider vinegar being particularly favorable. The numerous delicate flagella were demonstrated by laboratory's special method, from an eight-hours' growth on acid agar. Magnified 1,000 diameters.

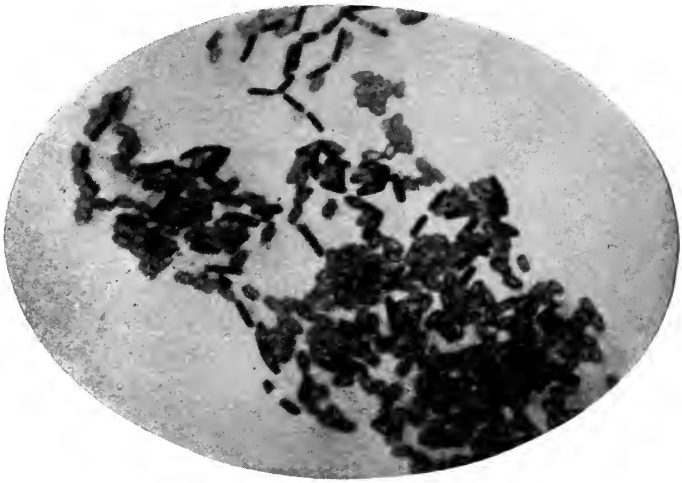


Plate 38. Bacillus Megatherium, showing Spores

Photomicrograph of the spore or seed forms of the pickle bacillus shown in Plate 37. The spores are large, thick-walled, and are formed in the center or near one end of the rod and are rapidly setf ree. Spore formation takes place rapidly in 24 hours. From an agar growth, preparation stained faintly with carbol fuchsine and photographed through the microscope, using Spencer 1-12 oil immersion objective and acetylene radiant. Magnified 1,000 diameters.

neighborhood of your factory may be laden with the spores of new varieties of hardy bacteria which have grown luxuriantly on the piles of waste in the yard. These find entrance to your cans and complications arise, goods undergo transformations, and you are tempted to blame Providence for your losses. The canners who are alive to the benefits of clean floors, machinery and tidy employes and who remove every obnoxious element out of the factory and yard are really the men who experience small losses from spoilage, and their careful methods create a care for the quality of the goods they pack and an interest in the selection of raw material. The result is that their goods bring the best prices, their establishments are open to the public and the whole moral effect of cleanly methods is apparent.

LACTIC FERMENTATION.

Lactic fermentation is accomplished by a large number of bacteria and is one of the most useful chemical changes. It is probably the earliest known form of decomposition, since milk gives a good example, and milk has been used as food from the time of creation and its use was no doubt familiar to the first inhabitants of the earth. In the early days of the microscope forms of life were noticed, chiefly those belonging to the wild yeast and mold fungi; but not until the time of Pasteur was there any definite understanding as to the true cause of lactic fermentation, for all theories previously advanced attributed the changes wrought in milk to spontaneous generation. Lister was probably the first investigator who obtained pure cultures of Bacterium lactis, a germ seldom found outside of dairies. Hueppe followed with a more careful investigation by means of gelatin plates and isolated several species of bacteria which were capable of setting up lactic decomposition. The principal organism discovered by him was Bacillus acidi lactici, which measures 1 to 1.7μ in length and 0.3 to 0.4μ in breadth, non-motile rods, usually in pairs. Bodies resembling spores are present, but they are not true spores, although so considered by some authorities. Casein is precipitated and carbonic acid gas is liberated at ordinary temperatures. Bacterium Prodigiosum, described under head of Chromogenic Bacteria, Chapter II, produces lactic acid.

Micrococcus acidi lactic (Krueger,) found as single or diplococci, is aerobic, forms lactic acid, and liquefies gelatin.

Bacterium lactic acidi, Bacillus lactis acidi, Bacterium acidi lactis, Bacterium limbatum lactis acidi, Micrococcus lactis acidi and Sphaerococcus lactis acidi, were isolated from sour milk by Marpman.

Bacillus Acidi Lactici, Hueppe

Origin.—Found in sour milk, also in fermenting vegetable matter.

Form.—Thick, short rods, two to three times as long as wide, usually found in pairs; but rarely found in chains or threads.

Motility.—It has no real motion, but has a marked Brownian movement.

Sporulation.—Round, terminal bodies have been observed, but are not spores.

Anilin Dyes.—Stain readily; so does Gram's method.

Growth.—Rapid and abundant.

Gelatin Plates.—The deep colonies are oval or round, yellow, finely granular, with sharp borders. The surface colonies spread and form thin plaques, having irregular wavy borders. The outer zone of the colony is almost transparent at first, showing markings which resemble the veins in leaves.

Stab Culture.—The growth on the surface is considerable, spreading rapidly; it is a thin, dry, white covering; growth along the puncture is slight. In old cultures bundles of crystals are formed along the line of inoculation, more especially at or near the surface.

Streak Culture.—On agar, a grayish-white, moist, spreading growth is formed. On potato, a brownish-yellow, slimy growth is formed.

Milk.—In sterilized milk, the lactose is converted, in part, into lactic and carbonic acids. Casein or curd is caused by the acid reaction thus produced. The change will occur only in the presence of air. Old cultures do not affect milk.

Oxygen Requirements.—It is a facultative anaerobe.

Temperature.—It will grow between 10° and 45° C., but grows best at about 30° C.

Behavior to Gelatin.—Does not liquefy.

Aerogenesis.—It forms gas in milk; also forms carbon dioxide and alcohol.

Pathogenesis.—It has no effect. Growth is stopped by 0.75% lactic acid. It produces lactic acid in the mouth (dental caries); also abnormal fermentations in the stomach and intestines. Lactic acid bacteria promote the growth of anaerobic bacteria.

Bacillus lactis (Bleisch), found in buttermilk and frequently in ordinary cream, is a large bacillus, forming spores of great resisting power. It is a facultative anaerobic actively motile organism, and converts glucose into lactic acid without the evolution of gas. The bacillus is about 1μ broad and 3 to 5μ in length and may be cultivated in agar plates, or on a substratum of cream of tomato soup. The specimen shown in Plates 40 and 41 was found in a can of sour tomato soup in which no gas had formed, the sugar having been broken up directly into lactic acid. On Agar and potatoes it forms a light gray coating.

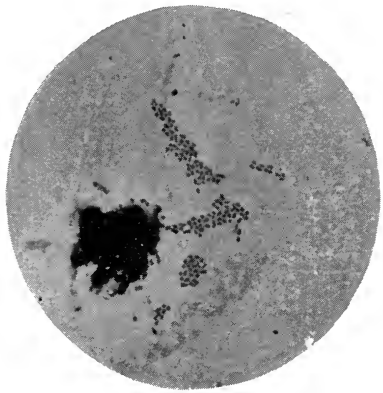


Plate 39. Lactic Acid Bacilli

Found on husks and in the juice of corn before processing. Causes souring of corn. Magnified 1,200 diameters.

The chemical equation for fermentation by this organism may be symbolized as follows:

$C_{12}H_{22}O_{11} + H_2O = 4\ C_3H_6O_3$
Grape sugar + water = Lactic acid (4 molecules).
$C_6H_{12}O_6 = 2\ C_3H_6O_3$
Glucose = Lactic acid (2 molecules).

The reactions are seldom so beautifully simple, however, for there are liberated by various germs, gases of different kinds which complicate the chemical equations, and the quantity of lactic acid is of course less than the expression given here. Lactic fermentation, even where pure cultures are employed, is accompanied by acids of a volatile nature.

A number of Pathogenic bacteria have the power of producing lactic acid when grown in milk; among these might be mentioned the Cholera bacillus, Bacillus of typhoid fever, Bacterium coli communis, Bacterium lactis aerogenes and Friedlander's bacillus of Pneumonia.

LACTIC FERMENTATION is utilized commercially in creameries to induce the *souring of milk*. For this purpose pure cultures of the very best bacteria are obtained from the laboratories and the cream is prepared and sown with these. Formerly the souring of cream preparatory to making butter was left to spontaneous action of the germs present in the cream, consequently a poor quality of butter was frequently turned out, due to injurious bacteria which were present with the desired species. Pure cultures of lactic germs are now made by and obtained from such stations as the Chris Hansen Laboratory of Little Falls, N. Y., and the acid generators are charged as follows: Skimmed milk to the amount of two per cent. of cream to be soured, is Pasteurized by

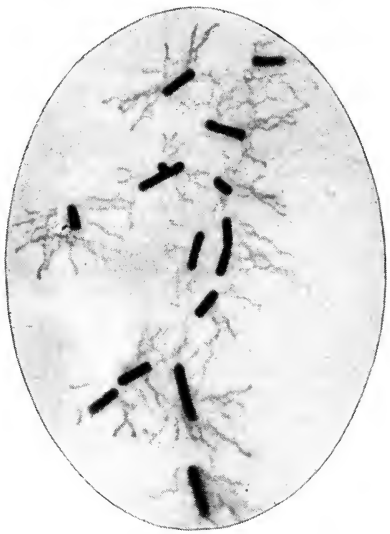


Plate 40. Aromatic Lactic Acid Bacilli, Flagellated

Photomicrograph of an aromatic lactic acid bacillus found on putrefying tomatoes. Magnified 1,000 diameters.

subjecting it to about 150° F., which kills many vegetating forms of bacteria. The skimmed milk is then quickly chilled, thus weakening the spores of the resistant species; then a pure culture of lactic bacteria is sown in the milk, which is kept in a temperature of 60° F. for twenty-four hours, in which time the lactic germs begin to vegetate very fast and gain the upper hand of the species previously weakened by heat. The acid generator is then ready to be added to the cream, which has been Pasteurized in the same way that was adopted for the skimmed milk. In another day the cream will have turned sour and is ready for churning. Butter

made from cream thus treated is very fine in flavor, and is purer and more wholesome than that made from cream which has soured spontaneously.

Milk is a fine medium for the invasion of Pathogenic bacteria, such as Tuberculosis, Typhoid and Diphtheria, etc., and all these are destroyed in the Pasteurization should they happen to be present.

A short description of modern butter-making in this connection falls under this head, especially the consideration of bacteria therewith associated. Butter is used extensively in various specialties which are canned and served in buffet cars, so the peculiar flavor of fine butter should be known to guide the canner in his selection.

I was kindly presented with a culture of bacteria used in the best creameries for inducing lactic fermentation in Pasteurized

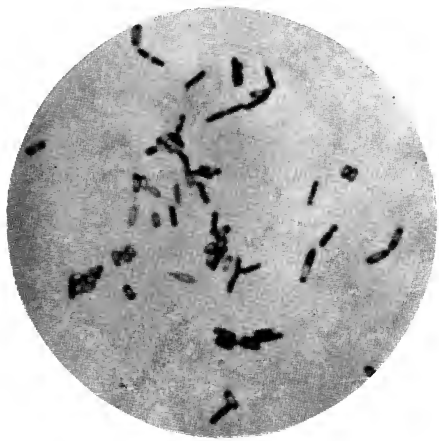


Plate 41. Aromatic Lactic Acid Bacilli

Showing rods and spores. Magnified 1,000 diameters.

cream by the Chris Hansen Laboratory, and here give a photomicrograph of the culture, which is named *"Startoline."* The culture shows the presence of Saccharomyces Pastorianus, and a coccus—described by W. Storch—which measures about 1μ in diameter. There is also a fission fungus, discovered by H. W. Conn in 1895, which is styled Bacillus No. 41. This is a non-motile rod about 1.1μ long, generally single, sometimes united in pairs, which grows best at 75° F. Cream is not coagulated and very little acid is formed by it.

The combined influence of these organisms is a pure lactic fermentation set up by the cocci and the fine flavor produced by the Conn Bacillus No. 41. There seems to be a difference between the flavor and the acidification, which may be described as follows:

The pure acidification gives a fine sweet taste to the butter, while the flavor or aroma has the characteristic of what is known as "grass flavor" or "June flavor," so marked in country butter made when the grass is green and tender. The Conn bacillus was isolated by him from a sample of milk he obtained from South America, and it is employed by a large number of dairies throughout the world.

Lactic acid bacteria play an important part in the preparation of the yeast mash in distilling, vinegar-making, and brewing. The preparation of the green malt for malt vinegar is interesting. The malt has many kinds of bacteria associated, some belonging to the butyric acid group. These are hardy types and these form spores of great vitality. The malt is mixed with water and heated to about 155° F., which kills all vegetating bacteria, but leaves

Plate 42. Micro-organisms in Lactic Acid Generator, called "Startoline."

the spores of the undesirable germs uninjured, which, if allowed to produce butyric acid, would prevent the yeast from accomplishing its part. In order to prevent these from vegetating, artificial souring is induced by lactic acid bacteria, which acidity is antiseptic to the butyric bacteria. The lactic acid fermentation is accomplished either by the aid of pure cultures planted in the mash or by maintaining the temperature at 120° F., which is about 20° higher than the optimum temperature for the development of butyric bacteria. At 120° F., the lactic acid bacteria flourish well, and lactic acid amounting to about 1 to 1½ per cent. is formed, which is ascertained by standardizing with normal alkali solutions, such as sodium hydroxid. The mash is then heated to about 155° F.,

which kills the lactic acid germs, also any other vegetated forms, and after cooling sown with pure cultures of yeast, which set up alcoholic fermentation without injury from the butyric and acetic acid groups.

The advantage of using pure cultures of lactic acid bacteria is great, for the reason that the spontaneous souring is apt to miscarry at times and forms of heat-loving bacteria often find their way into the mash along with the lactic acid bacteria from the air.

The pure culture now used is one discovered by Dr. Franz Lafar, of Vienna, from a yeast mash in the Lietzen Distillery.

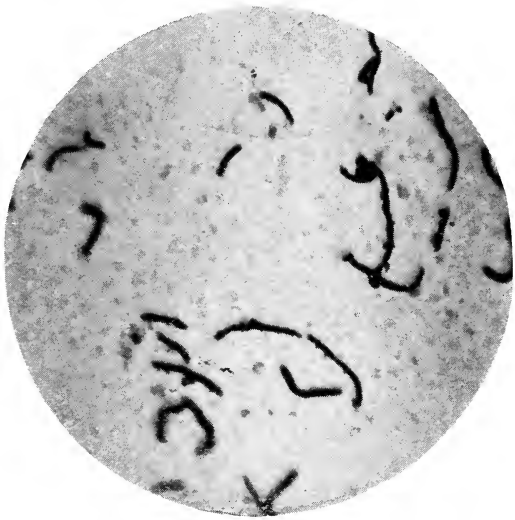


Plate 43. Bacillus Acidificans Longissimus

Magnified 1,000 diameters.

He named the germ Bacillus acidificans longissimus from the fact that it is very much longer than the ordinary lactic acid bacterium. This organism measures 1μ in breadth by from 2 to 20μ in length, and has the power of producing more lactic acid than any other germ so far discovered. A pure culture was obtained from the Berlin Experimental Distillery Station (Versuchsstation fur Brennerei), Germany, but was dead when it reached us.

By means of pure cultures of this organism, lactic acid may be produced at small cost compared to the price paid for the chemical preparation. Lactic acid is required for special industries, such as dyeing and color printing on fabrics.

Lactic fermentation takes place in the preparation of pickled meats, sauerkraut, pickles, white pearl onions, beets, cauliflower,

olives and various products which are put away for curing in brine. Only a limited amount of salt is used at first because salt has antiseptic power, causing plasmolysis of the germ cells. The lactic acid group does not suffer in the presence of limited quantities, and soon produces lactic acid in sufficient amounts to prevent the development of the germs peculiar to the various food products which are pickled.

The brine first added, ought to register about 75 on the Beaume salt scale, and as fast as the salt is absorbed more salt must be added until the fermentation is completed. At no time should the scale show less than 50 degrees. The brine will be found heavier at the bottom than at the top, so agitation is necessary or a pump may be used to lift the bottom brine up to the surface. If this be done once or twice a day good results will be obtained.

This method, while used extensively, is faulty from the fact that the lactic fermentation is set up spontaneously by germs from the atmosphere. The result is that harmful races of bacteria sometimes gain an entrance in company with the lactic acid germs, and unpleasant flavors are produced. Soft pickles, slimy cauliflower and discolored meats, are due to the admission of bacteria which break up cellulose and others belonging to the chromogenic group described in Chapter II. The bacteria which gain entrance with the lactic acid bacteria belong chiefly to the Subtilis group, and these thrive well even in the presence of considerable quantities of salt, and in the presence of lactic acid in limited amounts.

There is room for great improvement in the pickling of such products as mentioned; by first heating the raw material to a point where all vegetating forms will be killed—about 160° F.—and chilling, then by adding the proper amount of salt and sowing a pure culture of lactic acid bacteria, uniform results can surely be obtained.

PREPARATION OF SAUERKRAUT.

As we have stated, lactic fermentation plays an important part in the preparation of sauerkraut, the lactic fermentation being in fact the desired chemical change. Let us therefore look carefully into the process and gather a few facts that may lead to improvement of the quality and minimize the losses. After the cabbage is cored and cut it is put into large wooden tanks with salt sprinkled over each layer and packed firmly. Finally there is a top weighted down, and according to present methods fermentation is permitted to take place spontaneously. There are various spore-bearing bacteria and lactic acid germs throughout the whole tank. A gradual increase of temperature takes place and the various spores begin

to vegetate in proportion to the amount of air that is circulating. The temperature rises gradually until it reaches between 110° and 120° F.

At 100° F. the spore-bearing bacteria will gain the mastery and as more weight is put on the top the air is excluded and the temperature reaches about 120° F., which is the optimum point for the lactic germs, and the activity of the other class is impeded. At this temperature the lactic fermentation goes on until sufficient acid is formed to prevent decomposition by the other class. Now the improvement that suggests itself is this: Instead of depending upon the lactic acid germs in the atmosphere to find entrance to the cabbage, let a pure culture be sown throughout the tank and the temperature raised at once to 120° F. Lactic fermentation by the pure culture would begin at once, and the inimical germs would not be able to grow, which would give uniform results—a beautiful white sauerkraut with a delicate aromatic flavor. The temperature at the center may be tested by pushing a self-registering thermometer down from the top. After having once reached the proper temperature, pressure from the top can be regulated to keep it uniform.

There is no branch of the food product industry which suffers such severe losses as that devoted to *brining* and *pickling*, and there is great need of improvement over present methods to enable the manufacturers to depend on their processes. The installation of the laboratory where pure cultures of useful bacteria may be obtained is a great boon. There are many causes of spoilage in the salting houses; frequently the water is bad, due to minerals and injurious bacteria; different crops of raw material will vary in the number and character of bacteria infesting them, and inability to get proper fermentation at all times, makes the problem of securing uniform results difficult. No fixed rule can be laid down where dependence is placed upon spontaneous fermentation, and the quality secured depends largely upon the kill of the one who is doing the work. With pure cultures, however, there is very little chance to have poor quality if the material is good. There is a marked improvement in the quality of goods turned out in many industries due to the utilization of pure cultures of useful bacteria, and the manufacturers of food products will also gradually become interested.

Now let us condense the directions governing the lactic fermentation in brining:

FIRST, make liquids containing pure culture of lactic acid bacteria by stirring the culture into a quantity of distilled water.

SECOND, sprinkle the liquid over each layer of shredded cabbage, also the required amount of salt.

THIRD, increase the temperature to 120° F. and maintain at that point until the lactic fermentation starts, then control the temperature by pressure from the top.

ENSILAGE.—We have referred to the utilization of waste material, such as cobs, husks, peelings, strings and ends of beans, pea pods, etc. The quality of an ensilage made from these depends upon the care of the silo. The scientific principles are nearly the same as those outlined for brining except that the salt is not used. When the silo is filled, pressure is brought to bear to bring the temperature up to 120° F., which is the proper point favorable to the thermogenic bacteria, among which various races of lactic bacteria predominate. There are other varieties, however, which are heat-loving and some butyric and valeric acids are elaborated. By this method the loss of digestible albuminoids is great and compounds are formed which have no value in feed material for cattle. Ammonia and amide compounds are formed by the decomposition of albuminoids.

The undesirable features of this method can be entirely overcome by controlling the bacteria which are useful in the production of good ensilage. A pure culture of lactic acid bacteria, if sowed in the silo at the proper time and at the temperature favorable to their growth, will insure a valuable and marketable product for feeding farm stock.

The lactic bacteria are utilized in the tanning industry in the fermentation of the *plumping soak* and the bark liquor, which, of course, has no connection with the canning industry and simply mentioned here as a fact, interesting from a bacteriological standpoint.

Lactic bacteria, then, are useful in the preparation of various food products and the transformations accomplished by them are seldom inimical, as the great majority of them are easily destroyed at ordinary temperatures, but there is one, possibly two, varieties which are associated with milk and form spores of great resisting power.

PUTREFACTION.

Putrefaction is a term usually differentiated from fermentation by some authors because the material which undergoes putrefactive decomposition is albuminous, while the carbohydrates are changed by fermentation. Micro-decomposition, however, takes in putrefaction, as such transformations, whether accomplished in albuminous substances or in carbohydrates, are the result of the vital activity of bacteria; in fact, the same germs are frequently able to decompose either substance when planted under favorable conditions.

PUTREFACTION is accomplished in substances which contain carbon, oxygen, hydrogen, nitrogen, sulphur, etc., while fermentation has been restricted to substances containing only carbon, oxygen and hydrogen. The breaking up of albuminous substances is generally accompanied with disagreeable odors, which are noticed in such elaborations as indol and skatol where nitrogen and sulphur are combined. Sulphuretted hydrogen and the foul odor of dejecta are due to the cleavage of protein matter. Ammonia and the amines are the products of alkaline nature set free from decomposing proteins, while acids often pleasant in taste and flavor are formed by fermentation of carbohydrates. There are, however a number of bacteria which are peculiar to putrefaction and are seldom if ever found in carbohydrates.

The tearing down processes are therefore complete, fermentation splitting the carbohydrates and putrefaction splitting the proteins into simple elements. In this manner all dead matter, whether belonging to the vegetable or animal kingdoms, is reduced to simple elementary compounds and is not permitted to accumulate. Bacteria frequently cause death to both plant and animal life, but death may result from climatic changes or old age, or through renewing processes. The leaves of the trees are touched by frost and fall; the blossoms perish and the animal sheds his skin and hair and all nature is constantly putting off the old and renewing as fast as necessary. Death therefore is natural and not always caused by bacteria. One is apt to fall into the error that bacteria are primarily the cause of all dead matter, since they are known to be responsible for so many diseases, but close study of life will teach us that this is not true. How wonderful then is the plan of nature for the removal of the vast amount of organic matter that is thrown down all the time! Were it not for the bacteria the earth would soon be unfit for habitation and nature would be unable to furnish the elements necessary for removing worn-out matter.

The carbon (in decomposing matter) (combined in the molecules) is set free as carbonic acid gas, the hydrogen and oxygen combine and form water, the nitrogen and sulphur in the protein molecule form nitrates and sulphites and these elements are then in a simple state and can be utilized by vegetable kingdom directly and by the animal kingdom indirectly in building up new tissue; the vegetable kingdom using the carbonic acid gas in the atmosphere and the nitrates in the earth, and the animal kingdom using the starch, fats, sugar, etc., obtained from the vegetable kingdom. Thus we see that the vegetable kingdom is the source of nearly all food supply, to the herbivorous animals directly and to the carnivorous animals indirectly, while man is furnished by a vegetable diet directly and a flesh diet indirectly by the vegetable kingdom.

Vibrio Choleras Asiaticae, Koch (1884)

CHOLERA SPIRILLUM, COMMA BACILLUS; BACILLE VIRBULE (FR.).

Origin.—Found in the excreta of cholera patients, also in the intestines after death. Found several times in the water supply and milk.

Form.—Short, rather thick rod, having rounded narrowed ends, and varying from a straight rod to one bent in the form of a half circle; it usually resembles a comma, from which it derives its name of comma bacillus. If two cells remain attached the letter "S" is formed. When grown in liquid media under unfavorable conditions it may form long spirals. The bent rod is a segment of a spirillum and is called a vibrio. In old cultures peculiar involution forms develop.

Motility.—Actively motile, usually having at one end a single flagellum, sometimes two. Hanging-drop cultures should be developed at 37° —motion, spirals and involutions occur.

Sporulation.—So called Arthrospores. No resistant forms are known. Not true spores.

Anilin Dyes.—It is stained slowly. Carbolic fuchsin stains very well. Does not stain by Gram's method.

Growth.—At ordinary temperature it is fairly rapid.

Plates.—On gelatin plates kept at 22°, white or pale-yellow colonies, coarsely granular, with irregular rough border which is surroundel by a faint rosy hue, are formed. These colonies at first appear as small, white points; these gradually reach out to the surface, producing rather slow liquefaction, so that funnel-shaped depressions are formed. The colonies develop in fifteen to twenty hours. Entire liquefaction of gelatin occurs after several days. On agar plates at 37°, the large colonies present a peculiar, bright, grayish-brown appearance which is quite distinct from that of the common bacteria found in water and in feces.

Stab Culture.—In gelatin growth occurs along the entire line of inoculation. A funnel-shaped liquefaction with an air space above forms at the surface, the growth subsiding to the lower part. The lower part of the puncture is gradually widened by liquefaction; the growth settles to the bottom, and the entire contents of the tube are eventually liquefied.

Streak Culture.—On agar, a glistening, whitish growth is formed. It liquefies blood-serum slowly. On potato, kept in the incubator, a thin, grayish or yellowish-brown, somewhat transparent layer is formed. This resembles that of the glanders bacillus to some extent. Unless a mixed culture is used no growth is obtained at ordinary temperature.

Bouillon.—Rapid growth, especially in incubator, and a scum or pellicle is formed on the surface. Cultures twelve to twenty-four hours old display a reddish-violet color on the addition of sulphuric acid—the indol reaction—due to the formation of indol and nitrous acid.

Milk.—In sterile milk the growth is abundant, without much change; also in sterile water.

Oxygen Requirements.—Artificial cultures require oxygen.

Temperature.—Grows best at 37° C. Will grow at 15°-42° C. Killed at 50° C.

Behavior to Gelatin.—Liquefies slowly; old cultures, especially.

Immunity.—Subcutaneous or intra-peritoneal injections of the dead or living vibrio yield a serum which is anti-infectious; injections of the soluble toxin yield an antitoxic serum. The cell contents of the cholera vibrios render immune. Pfeiffer's reaction with the serum of convalescents or that of immunized animals or man (Chap. XIV).

Pathogenesis.—Rabbits are killed very quickly by intravenous injections. In guinea-pigs intra-duodenal injections or introduction of cultures into the stomach, previously alkalized, produce death with choleraic effects. The intraperitoneal injection of agar culture is fatal in the extreme to guinea-pigs; is attended with rapid fall of temperature. Subcutaneous injections of pigeons is not fatal. Typical cholera is produced in man by ingestion of cultures. The feeding of cultures to newborn rabbits and guinea pigs is usually attended with fatal results.

Infection.—Takes place along the alimentary canal, through water, food, contact with freshly soiled matter, etc. The bacillus grows in the intestines, and characteristic symptoms of intoxication are induced by the soluble poisons elaborated by it.

Putrid substances contain carbon and nitrogen in composition and the plants are unable to use them thus combined, so the putrefactive bacteria set in to decompose these combinations and the carbon is soon set free as carbonic acid gas, and the nitrates and nitrites find their way into the soil as ammonia, and this is utilized by plant life.

The putrefactive bacteria are called *saprogenic*, and by the old writers the *bacterium termo*, which is not a name signifying an individual species, but a class to which many allied species belong. The saprogenic bacteria may have the power to set up true fermentation when planted in substances containing sugar, starch, etc., but their true character is asserted in their ability to decompose albuminoid substances.

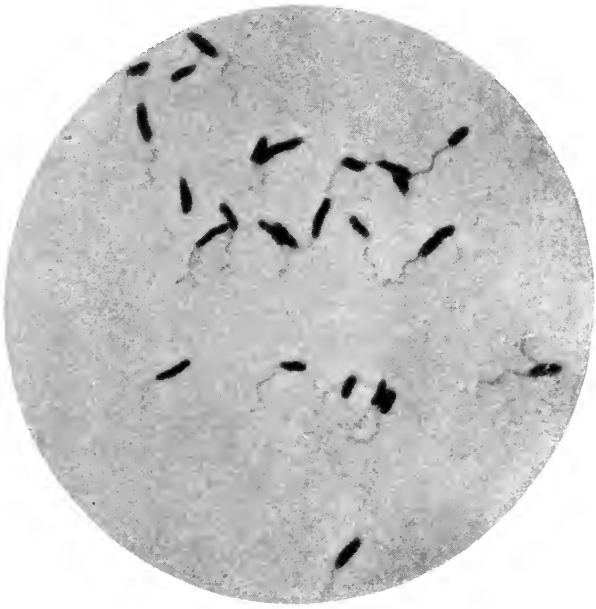


Plate 44

The decomposition of albumen is generally attended with bad-smelling gases. Some of these gases are so foul that the bacteria which produce them are hard to handle. The odor elaborated by one, called Proteus Vulgaris, is abominable.

The compounds which have such malodorous characteristics belong to the aromatic class. Several of them have been isolated by L. Brieger, M. Von Nencki, and E. Bauman.

Indol C_8H_7N is produced by quite a number of bacteria, and its presence forms a basis of differentiation of closely allied species.

It combines with nitrous acid as an imide to produce nitrose indol, which is red in color. This characteristic is brought out by adding Sulphuric Acid H_2SO_4 in the test. The Cholera Spirillum was the first pathogenic organism discovered which produced indol.

Skatol was isolated by Brieger in 1877 in human excreta, its presence being attributed to bacteria in the intestines; it is a very foul substance.

Sometimes, Phenol is a product of vital activity resulting from the decomposition of albuminoid substances; also Orthocresol and Paraocresol.

Methyl indol acetic acid is a very foul substance associated with degredation of albuminoids. It was isolated by N. Von Nencki from a culture of Bacillus liquefaciens magnus, growing in the anaerobic state.

Putrefaction is a common (phenomenon) in (imperfectly sterilized) canned goods of certain kinds, principally cans containing meats, meat extracts, vegetables containing albumen, and milk. This may be due to leaky cans or insufficient sterilization. The odors from swelled cans of corn, peas, beans and all kinds of meat are familiar to very canner of those products.

The greatest possible care in the packing of these goods is essential. In the process of putrefaction there are various ptomaines and toxic poisons formed, which sometimes cause considerable trouble. Whenever a case of poisoning occurs which the physician attributes to canned goods, the packer suffers to some extent, but the whole industry suffers more. Packers sometimes get very poor advice from incompetent writers and the following is a sample.

REPROCESSED LEAKS.

The following quotations are made from an article which appeared in one of the canners' journals in a series of articles published for the benefit of packers:

"The first paragraph is headed 'Defective Cans,' and reads: 'While piling out, some defective cans may be detected; these should have immediate attention. Open tip holes, repair cans, then retip and reprocess regular time. In some instances these may again be placed in the same grade of goods. When leaks are found after goods have stood several days, open tip hole, repair can, exhaust, tip and process regular time. Goods thus treated may be classed in a lower grade.'"

Such advice, scattered broadcast, is extremely dangerous. There is probably nothing outside of deliberately putting poison into food that would cause such dangerous stomach and intestinal complications as this practice.

In order that the packers may more fully understand the danger of putting out reprocessed leaks, let us look into the subject from various points of view.

1. There is danger of ptomaines forming in putrescible food.

2. There is danger of pathogenic molds and yeasts gaining entrance to acid foods.

3. There is danger of tin and lead poison.

4. The quality is extremely poor, therefore detrimental to the packers' reputation.

The first reason for not selling such goods is the *danger of ptomaines* having formed in putrescible material.

There are a number of bacteria freely distributed in the air, water and decomposing matter, which are capable of setting up putrefactive processes. These bacteria will not, as a rule, grow readily on raw material, but thrive luxuriantly on a great variety of cooked foods. Owing to their wide distribution in nature, physicians and some scientists have not taken true account of them and their power to produce ptomaines. A ptomaine is a complex chemical compound formed in several ways, principally as a product elaborated by certain bacteria belonging to the putrefactive class, and also to the pathogenic bacteria (which are the cause of diseases in man and animals and are parasitic on living protoplasm).

Not all ptomaines are poisons; only a few of them are so classed, but these few are either formed or excreted by a large number of bacteria. The poisons elaborated by the pathogenic bacteria are toxins which may be united with various other substances, and these are termed ptomaines; the real poison, however, is a toxin. Such bacteria as typhoid, tetanus, glanders, cholera, etc., all produce toxins, and these toxins unite with other compounds which may be extracted with ether and thrown down as ptomaines. The ptomaines commonly found in decomposing vegetables, meats, fish, cheese, milk, etc., are generally due to the more common and widely distributed organisms shown in the plates. There are also other varieties generally regarded as quite harmless which form ptomaines in very many different kinds of food, especially when forced to grow in an anaerobic condition, that is, where air is excluded. In the ordinary tin can this condition is very nearly complete, especially after fermentation has set in and carbonic acid gas or phosphuretted hydrogen has driven out the oxygen through a small leak. In such a condition the ptomaine bacteria are forced to obtain that very necessary element—oxygen—from molecules with which it is combined. The breaking down processes are therefore quite rapid and the chemical changes take place within very limited time, so that canned goods could thus be changed within forty-eight hours, or perhaps less.

Ptomaine poisoning is much more common than generally known. The evil effects are often experienced after meals, when cramps are followed by diarrhoea and severe headache. Sometimes these cases are quite severe, and the unfortunate suffer terrible pains with tremors, followed by coma, and even death. The cases on record are not a few, where whole companies of individuals have been stricken after banquets, suppers, picnics, parties, etc. Some of these cases have been thoroughly investigated, and the ptomaine responsible has been obtained from cultures of the bacteria found in the food. Usually in such cases the food does not, by any peculiar taste or odor, indicate the presence of an injurious substance. As we have previously stated, the chemical changes are rapid and the potmaines may be formed where *unperceived* decomposition is taking place. It usually happens that food containing ptomaines has been exposed, or worked over, and the task of defining the cause of such poisoning is often quite difficult.

I could mention a number of cases from all parts of this country and Europe, where families have been stricken and the poisoning was charged directly against canned goods by the attending physicians. In my writings on ptomaines, I have scored such decisions by physicians mercilessly, because I firmly believe that many of them jump at conclusions without proper investigation. Now permit me to say, if the packers should follow such advice as appears in the quotation in the beginning of this paper, no one with any respect for his integrity would dare to make defense against the charge that ptomaine poisoning is due to canned goods. Any physician who should happen to read that packers worked into salable packages goods undergoing decomposition would, without the least hesitation, cast the blame of such poisoning directly on canned goods, if any had been eaten by the unfortunate persons. In our laboratory work we have isolated from spoiled goods of many different kinds, various bacteria classed as common, some of them harmless to man when taken into the stomach, yet these very bacteria will produce ptomaines and toxins, especially when growing with other varieties. Even Bacillus Prodigiosus, the common bacterium which produces a red color on potatoes, bread, etc., a bacterium found commonly in the air, soil, water, decomposing material, and on the leaves, pods, vines and stalks of vegetation, will produce metabolic compounds when growing with other organisms, and these compounds have known toxic properties, fatal in some cases to animals, by subcutaneous and intraperitoneal injections. Bacillus subtilis and bacillus allii, the former a very widely distributed organism, the latter a bacterium found on decomposing onions, give rise to poisonous compounds in some cases. We have recently isolated a bacillus from a leaky can of mince

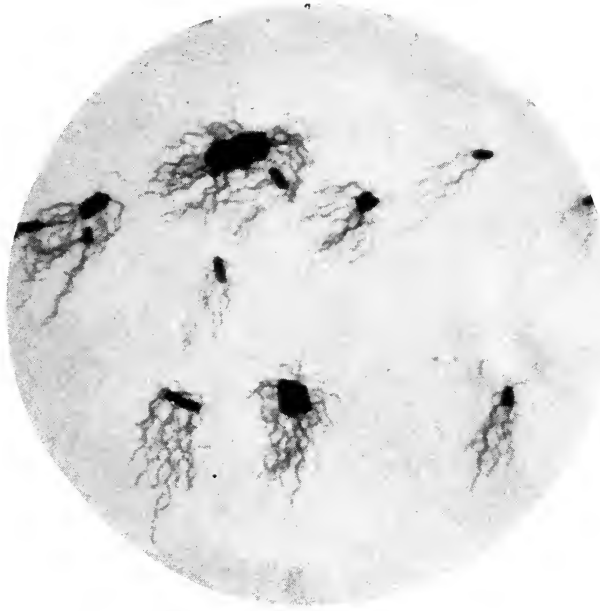


Plate 45. Ptomaine Bacillus from Mincemeat, showing Flagella

Photomicrograph of a bacillus found in perforated can of mince meat. This organism produces a ptomaine. The growth is similar to proteus vulgaris. It is endowed with numerous flagella, which are the organs of locomotion. It is actively motile. Isolated, stained by special method, mounted in xylol balsam, and photographed through the microscope. Magnified with 1-12 homogenous oil immersion lens and illuminated with acetylene radiant. Magnified 1,200 diameters.

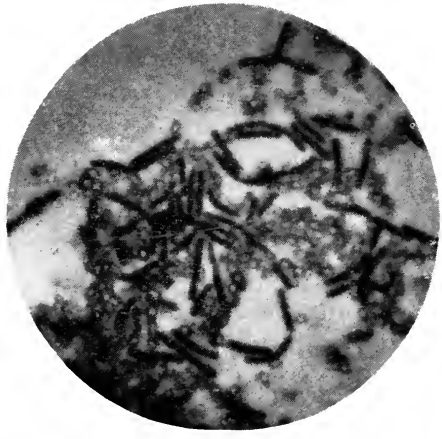


Plate 46. Ptomaine Bacillus from Mincemeat

Showing rods and spores. Magnified 1,200 diameters.

meat, which produces cholin, gadinin and trimethylamin, well known ptomaines. This mince meat was canned and a pie made with it made one person very sick, causing cramps, cold extremities and tremors.

1. Proteus Vulgaris, Mirabilis and Zenkeri and the colon bacillus are found throughout the alimentary tract in man and many animals, also on cooked foods undergoing decomposition. They are, therefore, quite common, but when permitted to grow in food will produce powerful ptomaines.

How is it, then, that we are not more frequently poisoned, if these are so common? Of the varieties mentioned one or more are taken into the stomach with various foods at every meal. We aim to eat only fresh food, which passes into the stomach where it is acted upon by the digestive enzymes, gastric juice, saliva and bile. These substances retard the development of these bacteria and destroy them in some cases. The food passes on to the intestines, where the acids are neutralized and the food is rendered alkaline. Here, then, is a favorable environment of temperature and alkalinity, so great numbers of bacteria grow rapidly, only to be cast out usually before any poisons are absorbed.

We can therefore readily see the difference between a partially decomposed food (containing these bacteria and their poisons) taken into the stomach, and perfectly fresh food, or food that has been kept free from the action of bacteria.

From this it is quite reasonable to suppose that some of the bacteria which form ptomaines will gain access to leaky cans and will form these poisons rapidly. I want to say here that after the ptomaines are formed they are not always driven out by cooking, in fact some of the most deadly ptomaines are active after cooking. The bacteria may all be destroyed, but their poison remains.

2. There is *danger of pathogenic molds and yeasts gaining entrance to acid foods.*

There are several molds that have been classed as pathogenic; prominently, Aspergillus niger and Aspergillus fumigatus. Molds grow well on acid fruits and vegetables and the products formed, while not considered deadly, might cause stomach and intestinal troubles. Several yeasts have been found which, when putrid, yield metabolic compounds of a basic nature, belonging to the amine group of ptomaines. (See Vaughn & Novy's "Cellular Toxins.")

3. *There is danger of tin and lead poisoning.*

It is a well known fact that tin and lead compounds are poisonous. Ordinarily, in canned goods, these are present in such small quantities that they are considered harmless. Several years ago the Bureau of Chemistry, Department of Agriculture, at Washington, gave this subject considerable attention. Some of the tin

Aspergillus Niger, Van Tieghem

Origin.—In putrid substances, in the lungs of birds, and acid foods.

Color.—Dark brown or black.

Mycelium.—Low and at first white, afterwards brownish or black.

Fruit-Organs.—The fruit hyphae are spherical, or flask—or club-shaped at the end which is covered with minute bottle-shaped bodies, radially arranged—the intermediate spore-bearers or sterigmae—from which extend rows of spores. These sterigmae are divided. The spores are brownish or black and spherical; are 3-5 μ in diameter.

Growth.—Slow.

Bread Flasks.—A low growth is formed which becomes very black.

Temperature.—Grows best at about 35° C.

Pathogenesis.—It gives rise to various ferments, diastatic, inverting, and others. The intravenous injection of spores in rabbits is not followed by as malignant results as with Aspergillus fumigatus.

plate examined at that time gave unfavorable results, but in general there were found only small quantities of the oxids of tin and lead.

When decomposition sets in, however, there are formed acids which attack tin plate and the lead in the solder most vigorously, and the gases throw them down in the form of insoluble oxids, which are poisonous. Canned goods which become swelled because of leaks have formed considerable acid and gas by the action of bacteria and when worked over must contain tin and lead in appreciable amounts.

4. *The quality is extremely poor*, therefore detrimental to the packer's reputation.

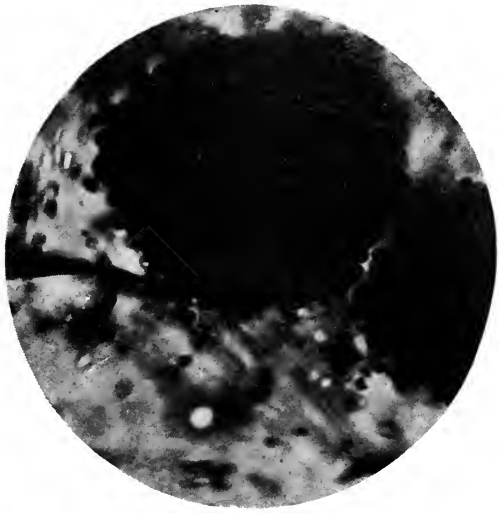


Plate 47. Aspergillus Niger

Photomicrograph of unstained mold Aspergillus Niger, which is often seen on food products, which it causes to ferment. It is pathogenic, producing substances deleterious to health. There are two large fruit pods shown. These are full of ripe black spores as conidia. Just below is one of the black threads of the mycellum. Magnified 600 diameters.

From all that we have written it must follow that goods sent out with such compounds formed in them will be very poor in quality; a second process would greatly injure the flavor in perfectly pure goods, but when poisonous compounds are also present one cannot imagine anything more detrimental both to the packer and the consumer. The evil results do not end here; the whole canning industry is assailed and maligned by the newspapers and hostile writers, and the innocent are made to suffer with the guilty.

Every can of goods you send out will probably reach a consumer who is looking for something good to eat; *every package will either make a friend or an enemy for your brand*, and when

you are packing your goods think of this all the time. If you have any cans which have suffered by accident in breakdowns of machinery or foreign matter having gained entrance, throw them away rather than run any risk of sending out goods of inferior quality.

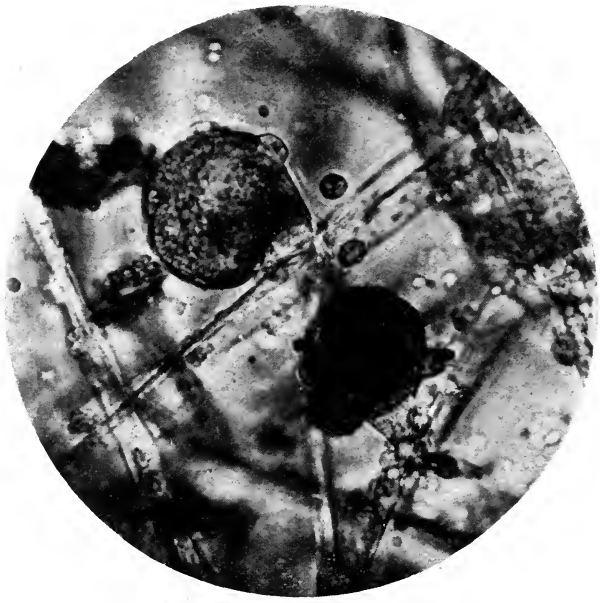


Plate 48

What disposition can be made of leaks, you ask? Cut them open, empty them and send the contents to the dump or wash them away in the sewer. However wasteful this may seem—do it—you will save more in reputation than you can ever gain by selling inferior and dangerous goods.

There should not be many leaks if there is a proper system of inspection. I find that out of a pack of over ten million cans one year I lost by leaks less than one-fifth of one per cent., or two out of one thousand. It is a good plan to have two inspectors, and women are, as a rule, quicker to see a leak than men. One of the inspectors is placed at a point as near the automatic capper as possible. As the cans pass this point all cap leaks, pin hole leaks, etc., are taken off the conveyor and patched. The other inspector

is placed at a point beyond the tippers and all tip leaks, buttons and cans with suspicious patching are sent back to the tippers.

The cans are conveyed into a tub of water where women gather and pile them top up, into process crates; and these women are trained to look for leaks as they handle the cans. A small premium for leaks found at this point is a good incentive for watchfulness and the cost can be made to fall on the inspectors and the tippers, according to plans carefully made. One man to patch, two tippers, two inspectors and four women to pile cans in crates will be sufficient help to properly care for 50,000 cans daily, and the leaks found in cans, imperfect tin and caps will not exceed, as I have said, more than two out of one thousand.

I stated previously that leaks should be cut open and the contents emptied. This should be done rather than to haul the swelled

Plate 49

Photograph of a can of tomatoes packed in 1884. This can is about the same diameter as a No. 2 can, but taller. The tomatoes after twenty years are in fine condition, just as nice as freshly canned stock. No "dating laws" required here.

cans to the dump. Strange as it may seem, in the large centers we find poor people who will take swelled canned goods home and eat them. In order to avoid any serious results it is better to dump the contents. Of course some packers may say that the poor should not do this, but in ignorance it is done, and the suggestion offered is worthy of consideration.

There are always a number of persons ready to take it up and rush into prominence with such bills as "Canned Goods Dating Bill" to correct the reported evils. There never was anything more absurd than a dating bill for canned goods, but should a ptomaine be present by any possible chance, a date on the can would not warn the consumer.

AGE DOES NOT AFFECT CANNED GOODS.

From Canner, March 2, 1905.

Plate 49 shows a can of tomatoes which was put up in 1884. The tomatoes are as good as the day they were canned. While the exterior of this can was very much rusted, the inside coating was perfect and was no doubt a very superior grade of tin plate. Age does not affect canned goods unless a perforation should happen to be made in the tin. So long as the air is kept away, the contents will remain in an unfermented condition.

From time to time there have been rumors of laws to declare the date of the pack on tin cans in various states, the idea being to limit the sale of canned goods to the year immediately following the pack. This would be very unjust because, as we say, canned goods are unaffected so long as the container prevents the germs from the air from gaining entrance. We have opened canned goods of various ages, ranging from five to twenty years, and have found that in every case where the tin was not perforated, the contents were perfectly good and tasted as well as the freshly canned product. American canned goods are the best and most wholesome food in the world.

CHAPTER V.

Decomposition Caused by Micro-organisms (Continued)

Putrefaction, Bacteria of. Ptomaines and Toxins, Pathogenic Bacteria and Their Action on Foods.

INTRODUCTION.

The study of putrefactive processes and the bacteria associated therewith leads up to the products elaborated by these groups. There are various products which are formed during the growth and multiplication of these bacteria, but the most important ones are ptomaines and toxins. These substances are not formed in canned goods unless there are leaks or they are imperfectly sterilized, although it has been charged against them quite frequently. In order to understand the nature of these substances and the bacteria which produce them, I have thought it advisable to describe the well known species.

The general character of contaminated raw material is carefully described, so that the manufacturer may be continually on his guard. A fair understanding of the subject may reduce to a minimum the chance of ptomaine poisons forming in manufactured foods of all kinds. The study of this subject will be interesting to the food chemist as well.

PUTREFACTION.

Sulphuretted hydrogen (H_2S) is a foul gas usually present during putrefactive processes. It has the sickening odor of rotten eggs and is produced by a long list of bacteria. Albuminoid substances are rich in sulphur compounds and the sulphur is easily liberated and combines with nascent hydrogen to form the gas. Bacteria cannot form this gas where sulphur is not present, which accounts for its absence where well known putrefactive bacteria are cultivated in certain nutrient media. The putrefactive bacteria use considerable sulphur in building up the protoplasm of their cells and the gas is formed only in small quantities in some cases.

Sulphur combines with ammonia and in some cases the gas is not liberated in sufficient quantities to be easily detected. This is interesting in connection with the production of ptomaines, as it shows that unperceived decomposition may take place in albuminoid substances and poison may be produced by bacteria in sufficient amounts to cause severe sickness, and even death, there being little or no evidence of any decomposition. The ordinary person depends largely upon his sense of smell to determine the decomposition of such foods as meat, fish, milk, cheese, etc., but it is generally the case where there is no perceptible decomposition that deceives because very few persons would eat any food that had a suggestion of putrefaction. The custom in some countries of permitting meats to age in order to soften the fiber must not be confounded with putrefaction.

Plate 50. Proteus Sulphurans

Photomicrograph of proteus sulphurans, a putrefactive organism which was isolated from decaying haddock. It forms great quantities of sulphuretted hydrogen. In all culture media the odor is abominable. It is actively motile and bores into the deepest layers of decomposing flesh. It has a wonderful array of flagella. Stained by our own special method and mounted in xylol balsam. Magnified 1,200 diameters.

Sulphuretted hydrogen is not easily perceived in the decomposition of albuminoid substances where nitrates are present, as these are reduced by the hydrogen to nitrites, both by aerobic and anaerobic bacteria. The presence of this gas is easily demonstrated in cultures of putrefactive bacteria by a simple and beautiful chemical reaction. Gelatin plates are colored Madeira yellow with (sodium ferri-tartrate 0.5 gram, water 50 c.c. and carbonate of soda added until alkaline); this combines with the sulphur and forms ferrous sulphate and a black halo or ring may be seen around

each colony of bacteria which produce sulphuretted hydrogen. It may be stated here that peptone added to the gelatin insures a more liberal production of the gas.

Nearly all pathogenic bacteria (bacteria which causes diseases of man and animals), form this gas; the typhoid bacillus produces sufficient quantities to be detected in a close room.

The bacillus which causes swine erysipelas produces more gas than any other I have met; a bouillon culture resembles yeast fermentation in the amount of gas bubbles liberated.

It must not be supposed that sulphuretted hydrogen is the only gas associated with putrefaction; there are various others; but its absence must not be taken as a guarantee that there is no putrefaction.

Putrefactive processes occur in the intestines of man, animals and birds. In man the large intestine is where this phenomenon takes place, and while putrefactive bacteria are not necessary, they find their way to that location, being introduced with food and water. The principal species is the Bacillus coli communis or Colon Bacillus, which resemble the Bacillus typhi abdominalis to such remarkable exactness that differentiation is difficult. The undigested albuminoids are attacked by this and other common putrefactive bacteria and those foul products known as indol, skatol, tyrosine, leucine, valeric acid, etc., are formed. Various poisons are formed also, but usually pass away without causing any dangerous complications.

There are a number of bacteria associated with putrefaction which produce ptomaines and toxic poisons in ordinary articles of food, such as meat, eggs, milk, ice cream, fish, cheese, etc., many of which have been isolated and their poisonous products have been separated. It must not be supposed that all putrefactive bacteria produce these poisons. They all produce enzymes of various kinds; some are poisons, while others are quite harmless. There are some ptomaines also which are not poisons, and to speak of all ptomaines as such, is a mistake which has only recently been cleared up. Putrescine and Cadaverine are two which were formerly considered as poisons, but recent investigation has proved the opposite.

Some of the pathogenic bacteria produce ptomaines which act as powerful poisons; many of the toxins have been separated and investigators are working to obtain these principles from all disease-producing bacteria. Some diseases formerly thought to be incurable have been successfully treated with the specific toxins of the bacteria which cause them. Among these might be mentioned, the anti-toxin obtained from cultures of Diphtheria Bacilli, which effects wonderful cures of diphtheria, and recently the tetano-toxin obtained from cultures of Tetanus Bacilli has effected cures of lock-

jaw when all hope of recovery by other means had been given up. These toxins, however, are not used in the pure state, which would prove fatal, but are attenuated by inoculating animals from which the weakened toxin is obtained in the serum.

Pathogenic bacteria will produce poisons on nutrient substances which have no albumen in their composition; these poisons are formed synthetically. These poisons have been named active albuminoids by the investigators who made the discovery. They resemble enzymes and have the power to decompose certain substances characteristically just the same as the ferment deposited by the yeasts. These active albumens are destroyed by 212° F. and become passive.

This discovery is valuable to the manufacturer of food products, since it throws a flood of light on many cases of poisoning blamed on canned goods. Many people eat uncooked meat in the so-called "cannibal sandwiches," smoked sturgeon and halibut, and in the same meal eat canned goods. If poisoning results, too often the blame is fastened on them, which causes considerable criticism of canned goods. Pathogenic organisms find their way into milk and on some raw vegetables, due to sprinkling with contaminated water, and the active albumen formed often causes severe cramps and even death. Cholera infantum is probably the result of contaminated milk; indeed, the common potato bacillus, Mesentericus Vulgatus, is capable of causing severe intestinal complications where milk containing it is given to infants in nursing.

There have been cases of ptomaine poisoning, however, which could possibly be traced to canned goods. There have been and possibly are today packers of canned goods who either are ignorant of the danger of canning unsound products or else they are unscrupulous. Such men cause a great deal of trouble to the industry as a whole, for all must suffer the severe criticisms of the press and must battle against unjust legislation. A National Canner's Association could put a stop to such practices.

I know personally one packer of meats and sausages who was arrested a number of times for attempting to use contaminated material. "No one would known the difference after it is canned," was his expression. There is no telling how much trouble this man may have caused, and it would hardly be supposed that there were no cases of poisoning where his goods were marketed. He used fictitious labels and covered his name so that it would be difficult to trace any complaints.

Such men should be forced to either put up wholesome goods under their own names or be compelled to get out of the business, and to accomplish this the law demanding the name of the manufacturer to be printed on his labels is wise and beneficial. *There*

would be no advantage in dating such goods, for unsound goods could not be detected by a date on the can.

There should be great care exercised in the selection of all raw material, especially such as contains albumen and is liable to putrefaction. There are means of knowing when raw material is good, both from general appearance and microscopical examination. Every canner should possess a good microscope, with an improved oil immersion lens.

There are a number of bacteria associated with meat poisoning which we will now describe, and these may be studied carefully, as the plates and descriptions will serve as a guide and reference.

One of the most common germs which produces a ptomaine is *Proteus Vulgaris*, found in putrefying meat. The rods are generally found in pairs. They measure 0.9—1.2μ in length, 0.4—0.6μ in breadth, but occasionally forms are seen 6μ long and they frequently form threads (100 μ long) when cultivated in nutrient gelatin. Spirilla, or curved threads, spirulina, or twisted threads, are also seen in gelatin cultures. Involution forms with swelled ends resembling dumb-bells and lemons are met with. The colonies on gelatin show the twisted and straight threads growing out from the center and these sometimes move out into the gelatin, becoming detached, so that the name of "Swarming Islands" had been given to them. The detached colonies sometimes resemble curious designs and figures and the name of "Bacillus figurans" has been given the germ on account of this peculiarity.

It is an actively motile organism on account of the large number of flagella it possesses which radiate from the whole surface of the cell.

The motion is seen to be in two directions; it turns on its long axis and moves rapidly forward at the same time. There is no spore formation and the cell life is easily destroyed by a moist temperature of 150° F.; in fact 135° F. kills it in a few minutes. It is a facultative anaerobe and grows well at 70° to 98° F., best at 75° to 80° F. It is stained well with carbol fuchsin, but Gram's method is negative.

On Gelatin Plates it forms small, round, yellowish colonies with thick centers, with the peculiarities before mentioned. The Gelatin is rapidly liquefied, both in plate and stab cultures.

On Agar a moist gray layer is formed, spreading rapidly over the entire surface.

On Potato a grayish coating forms.

Bouillon is uniformly clouded. Milk is coagulated and made faintly acid. It produces large quantities of sulphuretted hydrogen and forms indol; grows well in the presence of hydrogen and carbonic acid, and the odor from all media is abominable.

Proteus Vulgaris, Hauser

Origin.—It is very widely distributed; is commonly present in the putrefaction of animal proteins; has also been found in water, in meconium, in purulent abscesses, and in the blood and tissues of several cases of fatal putrid infection of the intestines.

Form.—Rods varying in length from short oval forms to those which are two to six times as long as wide. Grows in pairs, is usually bent; sometimes forms twisted, interwoven threads. Roundish involution forms are commonly found.

Motility.—Actively motile, with from sixty to one hundred flagella, arranged all over the surface.

Sporulation.—Not observed. Cultures are resistant to dessication and retain vitality for many months.

Anilin Dyes.—Stain readily. Will not stain by Gram's method.

Growth.—Very rapid.

Gelatin Plates.—Gelatin is rapidly and extensively liquefied. The colonies are of a yellowish-brown color, having bristly borders; in soft gelatin they have a tendency to spread over the surface, forming peculiar figures. Detached portions of the colonies may be observed to move about, which has gained for them the name of "swarming islands." A disagreeable odor is noticeable. They have an alkaline reaction.

Stab Culture.—Liquefaction extends along the entire line of inoculation; it is very rapid, and the whole contents are liquefied in a few days. The liquid, which is at first diffusely cloudy, clears up later and a flocculent sediment settles on the bottom. At the same time a grayish-white layer is formed on the top.

Streak Culture.—On agar, a grayish, slimy, rapidly spreading growth is formed. On potato, it forms a dirty colored, sticky covering.

Oxygen Requirements—It is a faculative anaerobe.

Temperature.—The optimum is between 20° and 24° C.; it grows very well in the incubator.

Behavior to Gelatin.—Liquefies rapidly.

Aerogenesis.—Hydrogen sulphide is formed.

Pathogenesis.—It has no effect in small doses. Toxic effects and even death, are produced by the injection of large quantities of living or filtered in rabbits and guinea-pigs. It is toxicogenic and sometimes may be pathogenic.

This and several related are included in the Bacterium termo of the older writers.

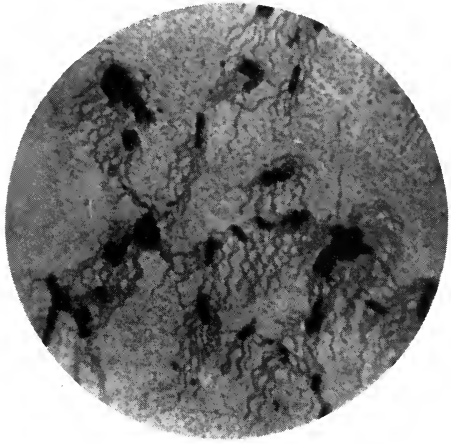


Plate 51. Proteus Vulgaris, Flagellated

Photomicrograph showing proteus vulgaris which produces a ptomaine. Magnified 1,500 diameters.

Plate 52. Proteus Vulgaris, showing "Swarming Islands."

Contact staining by the author. Magnified 75 diameters.

Pathogenesis. Animals, when subject to intravenous injections of Proteus cultures, die of acute enteritis and peritonitis, previously exhibiting typical symptoms of poisoning, such as bloody vomiting and diarrhoea, combined with severe tremors and feverish temperature.

The toxin is no doubt of a poisonous nature and is not destroyed by heat, even after the bacilli have perished. Patients suffering from "Weil's disease" are afflicted with this parasite, which may be obtained from the pus and urine.

PROTEUS MIRABILIS.

Proteus Mirabilis greatly resembles the organism just described, yet has distinct characteristics. The rods are various lengths, closely resembling vulgaris, but the threads are much longer, often attaining a length of 200μ. They are motile and possess many flagella. Spore formation is absent. The Gelatin plates and stab cultures are slowly liquefied. The deep colonies from curiously twisted masses or zooglea.

Large quantities of sulphuretted hydrogen are formed. Cultures show a decided indol reaction; milk is coagulated with faint acid reaction. Bacilli grow fairly well in an anaerobic state, either in hydrogen or carbonic acid gas. They are found in putrefactive processes and produce a toxin similar in its pathogenic effects to that elaborated by Proteus Vulgaris. The odor produced when cultivated on all nutrient media is very foul.

PROTEUS ZENKERI.

Proteus Zenkeri, called by some authors Bacterium Zopfii, is a bacillus 0.4μ broad and about 1.5μ long, resembling the two preceding species, but it is smaller and does not liquefy gelatin. It occasionally forms "Swarming Islands" like the other two and the other biological characteristics are similar. It is a peritrichous (many flagella) organism and produces a specific poison.

These three germs belonging to the Proteus family are remarkable for their rapid boring movements. They swarm through media which are solid enough to confine most motile bacteria. Our readers will be able from these descriptions and plates to recognize any of them under the microscope either in plates or in stained preparations. Other bacteria associated with Ptomaine and Toxic poisons.

BACILLUS BOTULINUS.

This putrefactive organism was discovered by Van Ermengem during an epidemic of poisoning from meats at Ellezelles in Bel-

Bacillus Mirabilis

Origin.—It is very widely distributed; is commonly present in the putrefaction of animal proteins; has also been found in water, in meconium, in purulent abscesses, and in the blood and tissues of several cases of fatal putrid infection of the intestines.

Form.—Rods varying in length from short oval forms to those which are two to six times as long as wide. Grows in pairs, is usually bent; sometimes forms twisted, interwoven threads, longer than Vulgaris. Roundish involution forms are commonly found.

Motility.—Actively motile, with from sixty to one hundred flagella, arranged all over the surface.

Sporulation.—Not observed. Cultures are resistant to dessication and retain vitality for many months.

Anilin Dyes.—Stain readily. Will not stain by Gram's method.

Growth.—Very rapid.

Gelatin Plates.—Gelatin is slowly liquefied. The colonies are of a yellowish-brown color, having bristly borders; in soft gelatine they have a tendency to spread over the surface, forming peculiar figures. Detached portions of the colonies may be observed to move about, which has gained for them the name of "swarming islands." A disagreeable odor is noticeable. They have an alkaline reaction.

Stab Culture.—Liquefaction extends slowly along the entire line of inoculation.

Streak Culture.—On agar, a grayish, slimy, rapidly spreading growth is formed. On potato, it forms a dirty colored, sticky covering.

Oxygen Requirements.—It is a facultative anaerobe.

Temperature.—The optimum is between 20° and 24° C.; it grows very well in the incubator.

Behavior to Gelatin.—Liquefies rapidly.

Aerogenesis.—Hydrogen sulphid is formed.

Pathogenesis.—It has no effect in small doses. Toxic effects and even death are produced by the injection of large quantities of living or filtered in rabbits and guinea-pigs. It is toxicogenic, and sometimes may be pathogenic.

This and several related are included in the Bacterium termo of the older writers.

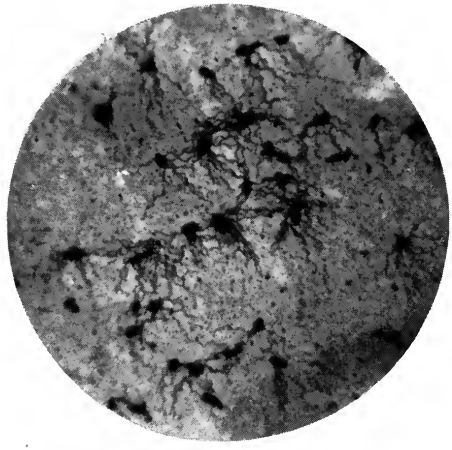


Plate 53. Proteus Mirabilis, Flagellated

Photomicrograph of proteus mirabilis, an organism which produces a ptomaine. Isolated from putrefying meat. Magnified 1,200 diameters.

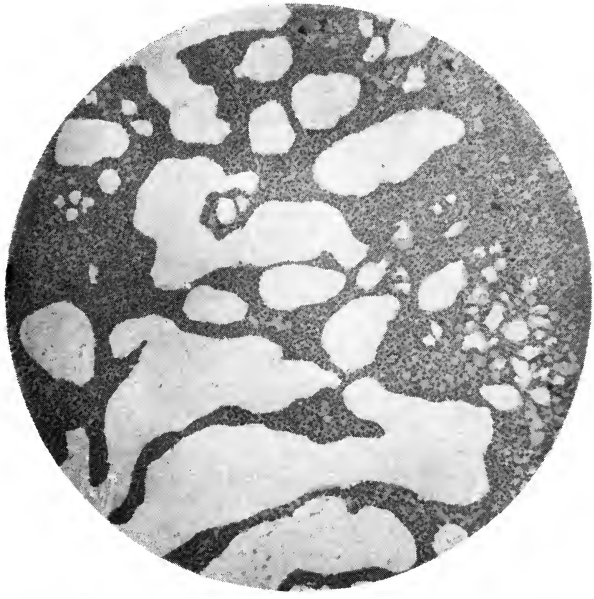


Plate 54. Proteus Mirabilis, showing "Swarming Islands."

Contact staining by the author. Magnified 75 diameters.

gium. The peculiar symptoms exhibited by the unfortunate victims of the botulinus group of bacteria, are nervousness of central origin, disturbances in the muscular system, the salivary and other secretions are suspended; difficulty in swallowing; hoarseness, mydriasis and ptosis, and death sometimes follows. The origin of "botulism" has been traced to contaminated salt fish, smoked meat, such as ham, preserved meats, blood and liver sausages, etc.

Bacilli botulini are seen under the microscope as large motile rods measuring 4 to 6μ long and 0.9 to 1.2μ in thickness, having rounded ends. Various involution (odd shaped) forms are sometimes seen. Threads are rarely formed, though sometimes three to ten rods will remain united.

When properly stained four to eight flagella can be counted, which give the bacillus a creeping motion. It stains readily with nearly all colors, and also by Gram's method (see article on staining) where alcohol is not used to excess.

It forms spores which are ellipsoidal and are situated in the ends of the rods (terminal spores). They are rarely median.

The vegetating forms are killed in boiling temperature 212° F., but the spores are more resistant. 250° F. kills them in a very few minutes.

Bacillus botulinus is anaerobic and grows well at 75° to 85° F., but will grow without sporulation at 98° F. Involution forms appear at blood temperature. The best artificial media for cultivation are made slightly alkaline with an addition of 2 per cent. grape sugar. It produces butyric acid and a toxic poison which can be precipitated in almost pure state by treating a bouillon culture with absolute alcohol, neutral salts and tannic acid.

Gelatin plate culture; round, transparent, brownish yellow colonies make their appearance in four or five days. These colonies have a thick, lustrous, granulated appearance, slightly liquefying the surrounding gelatin. When magnified sixty times the margins appear slightly irregular and radiating. In the stab cultures the course of the needle shows a white growth extending into the surrounding gelatin, which is liquefied with the evolution of considerable gas.

Grape Sugar Bouillon is clouded very much; in milk there is no coagulation and it remains unaltered.

Pathogenesis—Guinea pigs, cats, mice and dogs are killed by inoculation with the poison and also with the pure cultures. The nerve centers are greatly affected, principally the medulla oblongata, the ganglion of the hypolossal nerve, the dorsal ganglion of the vagus, the small-celled ganglion of the motores oculorum and brain nerves.

Bacillus Zenkeri

Origin.—Found in intestines of chickens; also in feces, water and putrefying substances.

Form.—Rods, two to five times as long as wide. Threads are formed; in gelatin they are often bent or twisted in peculiar shapes, resembling spirals. Coccus-like involution forms abound in old cultures.

Motility.—Actively motile.

Sporulation.—Involution forms are found which resemble spores. These are said to resist dessication, but are easily destroyed by heat, and stain readily with anilin dyes.

Anilin Dyes.—Stain readily.

Growth.—Rapid.

Gelatin Plates.—Delicate, cloudy patches of radiating threads are formed by the colonies, which show, under the microscope, in addition to these, numerous small, rounded bunches of cells.

Stab Culture.—There is a marked growth in the upper part of the tube, but none in the lower part; fine radiating lines penetrate into the gelatin, most deeply at or near the surface.

Streak Culture. On agar, a very thin, dry, grayish growth is formed.

Oxygen Requirements.—It is an obligative anaerobe.

Temperature.—Grows best at ordinary temperature. Will grow at 37° to 40° C. but has a tendency to develop involution forms and to die out.

Behavior to Gelatin.—Does not liquefy. No indol.

Pathogenesis. Produces a ptomaine.

Bacillus botulinus is sometimes found in putrefying meat, generaliy in the lean parts, seldom in the fat, and is able to flourish when the surface is covered with the aerobic putrefactive bacteria, which use up the oxygen and make the conditions favorable within the tissue for the development of anaerobic species.

This bacillus is particularly dangerous from the fact that it forms spores which will live through pickling and smoking processes and will afterward develop. Van Ermengem discovered the bacillus in a ham which had poisoned a number of people.

Albuminous foods, if properly handled in the raw state, will not suffer from this organism. Exposure to putrefaction is dangerous.

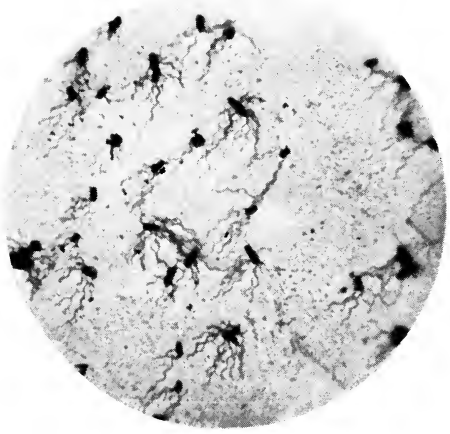


Plate 55. Proteus Zenkeri, Flagellated

Photomicrograph of Proteus Zenkeri, an organism which produces a ptomaine. Isolated from putrefying meat in a leaky can. Magnified 1,200 diameters.

BACILLUS ENTERITIDIS.

Bacillus enteritidis belongs to the Coli group. It was discovered in 1888 by Gartner in a meat poisoning epidemic. He obtained it from the tissue of a cow, which died of mucous diarrhoea, and from the spleen of a man who had died from eating its flesh. It is morphologically identical with the Bacterium Coli Dysentericum, which has been proved to be the cause of epidemics of dysentery.

Bacillus enteritidis has been found to be the cause of many cases of poisoning from meats; it appears as rods 2 to 4μ long and 0.4 to 0.6μ broad. The ends are rounded and are refractive, especially when the organism is cultivated on gelatin and examined in the hanging drop culture. It possesses five to ten flagella and

Plate 56. Bacillus Botulinus, showing Flagella

An organism which produces a poisonous ptomaine.

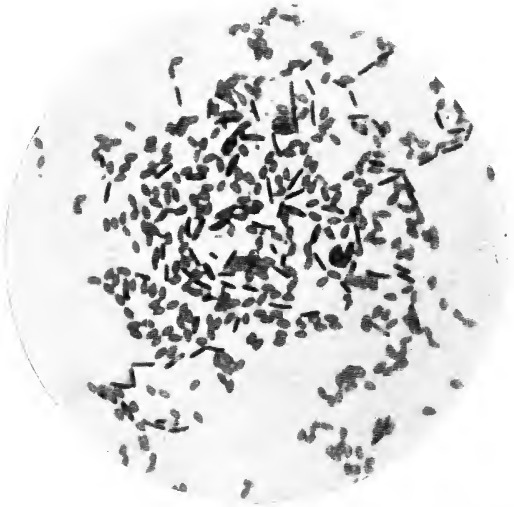


Plate 57. Bacillus Botulinus, showing Rods and Spores

Magnified 1,200 diameters.

is motile. (This is denied by Lehman and Neumann.) Their error is possibly due to investigating old cultures. Cultures twenty-four hours old show a true independent motion and flagella can be demonstrated by the method of staining (described in Chapter III). The rods stain more deeply in the middle than in the ends, due to their richness in fat of alkaline nature. An even stain may be made by first treating the germs with a 20 per cent. solution of Sulphuric Acid, H_2SO_4, which neutralizes the alkaline, and the stain takes quite readily afterwards. Gram's method is negative.

It grows well in temperatures ranging from 70° to 100° F., best at 98° F. Gelatin Plates show the superficial colonies as thin, almost transparent films, and there is no liquefaction of the gelatin, either in plates or Stab Culture. There is very little odor in any of the culture media.

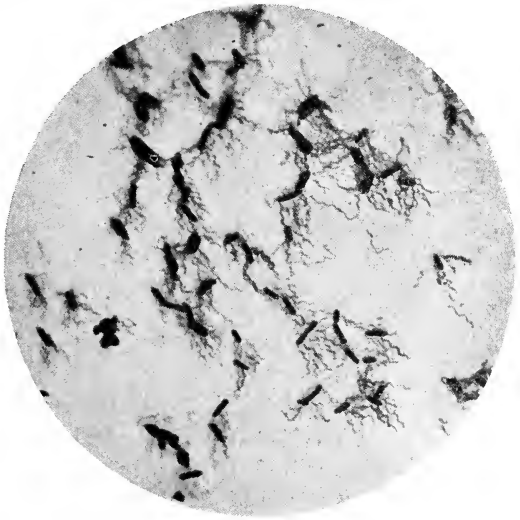


Plate 58. Bacillus Enteritidis

Photomicrograph of an organism which produces a deadly poison. Magnified 1,000 diameters.

AGAR PLATE cultures show a gray film, almost transparent. Bouillon cultures is uniformly clouded. Grape sugar bouillon is acidified with the evolution of Carbonic Acid Gas and a combustible gas similar to marsh gas. Milk Sugar Bouillon is not acidified, but the same gases are formed less abundantly.

MILK is not coagulated nor changed chemically. Young cultures do not form indol; old cultures show slight traces. It is an aerobic organism, facultative anaerobic in the presence of grape sugar.

PATHOGENESIS.—When this organism grows on meat a powerful ptomaine poison is found which heat does not destroy. Even the broth from such meat will contain the poison. The poison may be precipitated with absolute alcohol, tannic acid and neutral salts, if acid is present.

Live cultures of the germs when inoculated subcutaneously into small animals cause death in three to eight days. Intravenous injections of the ptomaine result the same. Infected meat fed to animals causes intense gastro-enteric cramps and death. These symptoms are the same in man. This form of meat poisoning follows the consumption of meat obtained from diseased animals.

Owing to the imperceptible decomposition set up by bacillus enteriditis it is indeed a dangerous organism. It may be present in ham sausage and fresh meat without any outward indication of its presence. The milk obtained from diseased animals may contain the germs and to all appearances it may seem good. Canners of meat are menaced by such an organism and need inspectors to examine all meat that is used. The same applies to the canners of assorted soups, where meat is used for the stock. Manufacturers of these goods ought to have a man to inspect and examine microscopically all meats used.

Not all cases of gastro-enteric disturbances prove fatal or even serious. Nearly every person, at times, is subject to these, and it is safe to say that ptomaines are responsible in fully one-third of the cases, but the causes are not understood and are passed by. It is only when severe cases are brought to the attention of the public that any criticisms are published. Ptomaines are most frequently formed in raw material bought in the open market. Eternal vigiliance is the only safeguard for the manufacturers who use albuminous material. It is well to state here that such disturbances as we have described may result from other causes; overheating incompatible foods, and drinking too much liquor often cause complications of this nature.

BACILLUS MORBIFICANS BOVIS.

This organism was isolated by Basenan from the tissue and spleen of a cow which died of puerperal fever. It is biologically and morphologically the same as the Bacterum of swine cholera. Ostertag found that ptomaine poisoning was produced where the flesh of cows affected with puerperal fever had been eaten.

It is an actively motile organism, having 8 to 14 flagella, the rods measuring 0.3 to 0.4μ broad and 1 to 1.2μ long, generally united in pairs. (It resembles the typhoid bacillus). Spore formation has not been observed. It stains readily with all ordinary dyes, but Gram's method is negative.

Colonies on gelatin and agar resemble those of Bacterium coli, but have a more granular appearance. Gelatin is not liquefied. Agar stab cultures have white tufts. Bouillon is clouded and a thin pellicle is formed on the top. Cultures on sterile potato are yellow and moist and do not darken. Milk is not coagulated. Grape sugar is slightly fermented with two gases liberated, Carbonic acid and Hydrogen. Cane sugar is not fermented. Indol and Phenol are not formed. The bacillus is killed at boiling temperature and even at 160° F., but the ptomaine is not so destroyed. Both the meat and the milk from diseased cows will retain the poison. Very small animals are killed by inoculation or from eating the flesh; dogs and cats are not affected. The same precautions we have mentioned previously will prevent poisoning from this saprophyte.

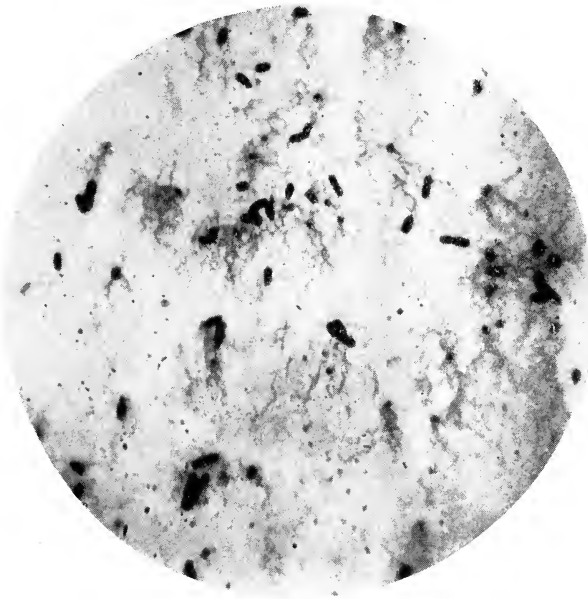


Plate 59. Bacillus Morbificans Bovis, Flagellated

Magnified 1,000 diameters.

BACILLUS MALLEI.

This is the bacillus which causes glanders and produces a toxic poison which is fatal to some animals whose flesh is used as food, viz., sheep and pigs. The horse is very susceptible and man also, but our object is to show its association with meat poisoning. The bacillus was dicovered by Loeffler and Schutz and occurs as non-motile rods 2 to 3μ long and 0.4μ broad, showing bright shining

Bacillus Mallei, Loeffler and Schutz, (1882)

GLANDERS; MORVE (FR.); ROTZ (GERM.), MALLEUS (LAT.).

Origin.—It is found in the nodules, ulcers, discharges, etc., of glanders.

Form.—Straight or slightly curved rods, with rounded ends, shorter and thicker than the tubercle bacillus. Usually single, but may grow in pairs or in short threads.

Motility.—Marked Brownian motion.

Sporulation.—Bright bodies, considered by Loeffler as the first indication of degeneration, are often found in the cells, but real spores have not been found. It is not very resistant to dessication.

Anilin Dyes.—It stains unevenly and is rapidly decolored. Carbolic fuchsin, alkaline aniline gentian violet, or anilin fuchsin, stain well, especially when warmed. Does not stain by Gram's method.

Growth.—Grows best at relatively high temperature. Rapid. Grows best on glycerin agar.

Plates.—Excellent colonies form in a day or two on glycerin agar at 37°. The colonies are round, grayish and glistening, having smooth sharp borders and with granular contents. Colonies cannot be obtained on gelatin, as a rule.

Stab Culture.—Develops very slowly in gelatin; can be made in glycerin agar.

Streak Culture.—On glycerin agar a thick, moist, slimy growth is formed. On potato it forms a thin, transparent, amber-colored growth, which later becomes a reddish-brown. On blood-serum yellowish, transparent spots are formed; these later run together, yielding a slimy, whitish growth.

Bouillon.—Grows readily and abundantly, with diffuse cloudiness; ring of slime on the surface. Mallein is the filtered bouillon of the glanders bacillus. It is analogous to tuberculin.

In milk and acid reaction is produced.

Oxygen Requirements.—It is a facultative anaerobe.

Temperature.—Grows best at about 37° C. Does not grow readily above 42° or below 25° C.

Behavior to Gelatin.—There is very slight growth at first, but may become accustomed to growth at room temperature later.

Attenuation.—Takes place rapidly when grown on artificial media. If the bacillus is not frequently passed through an animal the virulence is lost and the organism may die out.

Immunity.—Intravenous injections of small amounts of bouillon culture render dogs immune.

Pathogenesis.—Man, horse, ass, goats, cats, guinea-pigs and field-mice are highly susceptible. Cattle, hogs, ordinary and white mice are immune. Dogs, rabbits and sheep are slightly susceptible. White mice fed with phloridzin become susceptible. On inoculation susceptible animals develop typical glanders. In guinea-pigs death will result in four to eight weeks. Field mice die within a few days. Enlarged lymphatics, nodules in liver, spleen, etc. Bacilli are present.

Infection.—May occur through wounds—inoculation glanders. A man was accidentally and fatally inoculated with a pure culture in one instance. The usual source of infection in horses is probably along the respiratory tract.

granules, which are not spores, although some authors have erred in so stating. The rods generally appear singly with rounded ends. Pairs are sometimes seen and rarely threads. Sometimes branching threads are seen in old cultures. The bacillus stains somewhat difficultly with ordinary dyes and Gram's method is negative. It greatly resembles the Diphtheria bacillus in staining on account of the granules before mentioned.

It is aerobic, facultative, anaerobic; grows at any temperature between 77° F. and 108° F., best at blood heat, on 5 per cent. glycerine agar.

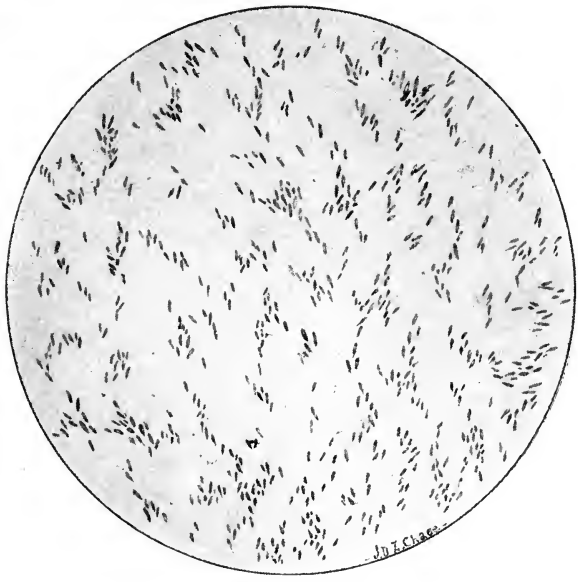


Plate 60. Bacillus Mallei

Photomicrograph of Bacillus mallei, showing bright, shining granules, resembling spores. Stained with Loeffler's menthyline blue. Magnified 1,000 diameters.

A characteristic growth is exhibited on sterile potato at 98° F., when moist amber-colored patches make their appearance; these deepen to a red brown and become thicker, sometimes forming interlaced threads. The surface surrounding the growths turns quite dark. Acid is produced in media containing grape or milk sugar. Bouillon is made slightly cloudy with a sediment (and a trace of indol). Milk is coagulated slowly and separated into casein and clear whey, owing to the production of acid. No gas is formed from carbohydrates.

The Bacillus Mallei is destroyed at 212°F. in a few minutes, but the specific poison is still virulent. This toxin has been separated by filtration and is called *Mallein* and is analogous by the tuber-

culin of the tubercle bacillus obtained by Dr. Koch of Berlin. Mallein is used in cases of glanders as a cure and gives good results in early stages of the disease. It is also used as a means of diagnosis of glanders in animals.

Sheep and pigs are susceptible and should these be slaughtered and the meat be consumed, severe cases of poisoning would result. The poison would remain virulent in the meat even after cooking or canning. Pork is used largely in the preparation of canned pork and beans. Great care and judgment should be exercised in the selection of the pork. Good pork has a firm and healthy appearance in the pickle and the presence of bruises, soft spots or disease growths should immediately condemn it as not fit to use. Do not cut out the bad parts and use the piece, but refuse the whole tierce if there are any such characteristics. Some packers are tempted to use inferior pork, because the price is a few cents less per pound. Use only select pork and the general appearance will be a good indication of its wholesomeness. Of course, the microscope should be used constantly to determine the character of the albuminous material used in canning, and this applies to the examination of pork particularly, in the detection of bacteria as well as the deadly trichinae.

Our readers have now become acquainted with some of the germs which produce ptomaines and toxins, but there remain a few belonging to the Pathogenic class, which are at times epidemic and are familiar to everyone as the cause of special diseases. Their description and biological characters will be interesting, not only in connection with out subject at hand, but also from a bacteriological standpoint.

OTHER PATHOGENIC BACTERIA ASSOCIATED WITH PUTREFACTION AND FOOD POISONING.

There are a number of bacteria which are pathogenic but are able to grow on certain food and in water, where they form poisons which are nearly related to ptomaines. Some of them form toxins which act as poisons and these may be formed in food which seems to be wholesome in every respect. It often happens that the bacteria are carried in the food and cause epidemics of disease, and the description of several common varieties will be interesting to the student of foodstuff for this reason.

TYPHOID BACILLUS.

This organism was discovered by Eberth in the internal organs of persons who had died of typhoid fever. The bacillus was observed by Dr. Koch in the typhoid abscesses and he made photomi-

crographs of it. The typhoid bacillus is particularly interesting to the student of bacteriology and may be obtained in pure cultures in the manner we shall describe later (article on plate culture).

It occurs as short plump rods with rounded ends, singly, sometimes in pairs, and when grown on potato in threads. It measures from 1 to 3μ long and 0.5 to 0.9μ broad. It grows on agar well at 98° F. and this culture gives the most satisfactory results for flagella staining. Bright shining spots are seen at the ends of the bacillus and these were thought to be spores by Gaffky, but such is not the case.

It is actively motile, possessing eight to eighteen flagella, which give the short rods a wonderfully rapid motion. It travels very fast, turning somersaults. The flagella are long and wavy and grow out from the whole surface of the bacillus. The isolation of the bacilli and the proper staining of their flagella is a beautiful bacterilogical test of skill, and when successfully achieved fits the student for the most complicated work. Plate 61 is photographed from a slide prepared from an agar culture and the flagella are stained by the author's method.

The typhoid bacillus stains well with carbol fuchsin and gentian violet, but with other dyes the rods do not stain as readily as many other germs. Gram's staining method is negative. The bacilli grow in clumps in the tissues and spleen and when stained thus should remain for one day in Loeffler's methylene blue or Ziehl's carbol fuchsin and then washed in distilled water. Methylene blue fades after a time, so the carbol fuchsin or gentian violet is preferable.

The typhoid bacillus is aerobic, and does not liquify gelatin. It grows well upon nearly all nutrient media at room temperature, but most luxuriantly at blood heat in an incubator. It will grow as an anaerobe also and thrives fairly well in the presence of CO_2 (carbonic acid gas).

BOUILLON CULTURE.—Bouillon is clouded, becoming slightly acid, and there is a quantity of sediment formed from which Brieger obtained in 1884 a ptomaine which he named Typhotoxin $C_7 H_{17} NO_2$. This ptomaine forms in considerable quantity in a test tube kept at 98° F. for one week. This ptomaine is strongly alkaline. Typhotoxin produces salivation, rapid respiration, dilation of the pupils, diarrhoea and death when given to small animals such as guinea pigs. The experiments have not been tried on man, but Brieger believes that the specific action of the typhoid bacillus in tpyhoid fever is due to the production of the ptomaine.

MILK CULTURE.—The bacillus grows well in milk and forms some acid, but does not cause coagulation, hence its presence is not easily detected. The ptomaine is formed, however, and has

Bacillus Typhosus, Eberth, Koch (1880)

BACILLUS OF TYPHOID FEVER; KOCH-EBERTH'S BACILLUS.

Origin.—It was first obtained from the spleen and lymphatic glands of typhoid fever cadavers. It is present in the blood in small numbers; also in the feces and urin of typhoid patients.

Form.—Rather large rods, two to three times as long as wide, with rounded ends. The length depends upon the medium on which it grows. On agar the rods are short; on potato long threads appear. Involution forms.

Motility.—Actively motile, with numerous lateral flagella; fine giant whips. It may show very little or no motion on prolonged artificial culture.

Sporulation.—Round or oval terminal bodies occur in potato and agar cultures grown in the incubator for several days. They will not double stain, and the bacilli which contain them are very susceptible to heat. These are not true spores, but little masses of condensed protoplasm. The bacilli are very resistant to dessication, and may retain their vitality for months.

Anilin Dyes.—Do not stain well. Carbolic fuchsin stains very well. Gram's method will not stain.

Growth.—Is less rapid than that of the Colon Bacillus. It grows slowly at 16-18°.

Plates.—On gelatin plates the deep colonies are small, round or oval, yellowish and finely granular, with sharp border. They sometimes show a dark portion or ring in the center. A protuberance may frequently be seen on the border, which is surrounded at times with delicate fibrils. The surface colonies form a spreading, almost transparent film, marked with delicate, branching lines, and having an irregular, wavy border. There is no liquefaction.

Stab Culture.—Growth is abundant along the entire line of inoculation; is especially so on the surface, spreading there as a thin, grayish white covering. Acids are produced which cloud the gelatin.

Streak Culture.—On agar and on blood-serum a white, moist growth is formed. On potato, a moist, invisible layer is formed. On alkaline potato the growth is yellowish; not characteristic.

Bouillon.—Is slightly cloudy, not so much so as with the Colon bacillus. There is very little deposit; scarcely any ring or film. Remains clouded for a long time. It will not grow in bouillon containing 20 c. c. of N HCL or 50 c. c. of N NaOH per liter, unlike the Colon bacillus. No indol is produced. In Uschinsky's medium there is no growth.

Milk.—Is not coagulated. No gas is formed in glucose media; no acid in lactose media.

Oxygen Requirements.—It is a facultative anaerobe.

Temperature.—Grows best at 37° C.; also grows well at ordinary temperature. Is killed by exposure to moist heat of 60° C.

Aerogenesis.—No acid or gas production on lactose media.

Behavior to Gelatin.—Does not liquefy.

Immunity.—Injections of dead or living cultures yield an anti-infectious serum; injections of the toxin yield an anti-toxic serum. The serum in the former case will give Pfeiffer's reaction with the Eberth, but not with the Colon bacillus.

Pathogenesis.—Rabbits are usually killed by intravenous injections. It is usually fatal to guinea-pigs when injected into the duodenum or the peritoneal cavity or when introduced into the previously alkalized stomach. Guinea-pigs are killed bp subcutaneous injections also. Abscesses are produced in dogs and rabbits by the same method of infection. It may produce abscesses in man. Cultures killed with chloroform or by heating for one hour at 54° are fatal to guinea-pigs in doses of 3-4 m. g. per 100 g. body weight.

Infection.—Takes place commonly through the mouth by means of water, food, soiled articles, etc. It may be transmitted through the air as fine dust. Carried by flies and other insects.

Plate 61. Typhoid Bacillus Flagellated

Magnified 1000 diameters

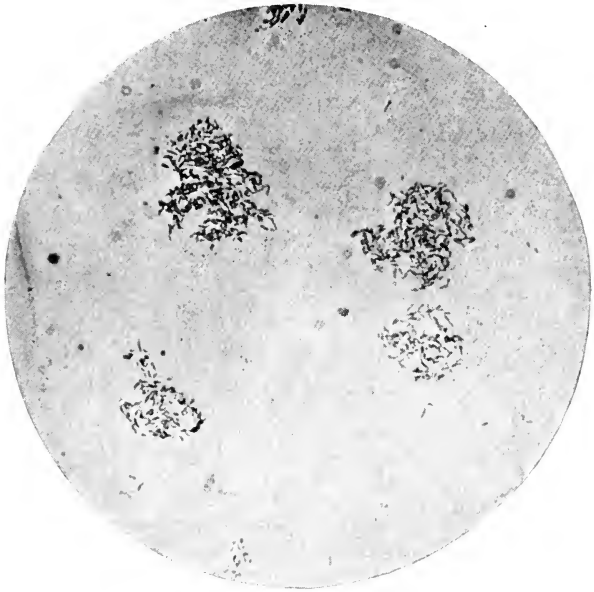


Plate 62. Typhoid Bacillus, showing Agglutination

Magnified 500 diameters.

been the cause of severe poisoning I have no doubt. Milk is a great carrier of the disease and many epidemics have been traced to it. The contamination is usually made through the water used in rinsing the pails, especially if the water is obtained from wells or springs where the bacilli have found entrance. Many farm wells are close to closets and cesspools and become contaminated from the faecal matter of typhoid patients. Epidemics have been traced directly to this source and there are official records of a number. In 1870 Ballard investigated the epidemic in Islington, where 167 people contracted the disease, and the investigation led to the discovery that the milk used by these people was obtained from a farm where the well was contaminated by rat holes connecting with the closet. The farmer had a case of typhoid fever and the well water was used to rinse the pails.

The fever broke out in the prisons at Strasburg in 1890, and the disease was traced to the milk supply, which came from a place where the fever was epidemic. Rowland, in 1892, found the living bacteria in what is known as "Dahi," which is an Indian milk comestible.

Since milk is one of the principal ingredients of so many fine food products, especially of the cream soups, it is perhaps wise to advise the use of "Pasteurized" milk, for this method destroys all such germs as the Typhoid bacillus long before they have opportunity to multiply and form ptomaines. The great danger from using raw milk lies in the possible contamination by disease germs, and while these will be destroyed in the sterilizing process, the poison still remains in certain quantities, and may cause sickness varying in violence according to the amount of poison present. Perhaps the amount of poison will be so slight as to cause no apprehension, yet the food product will not give satisfaction.

POTATO CULTURE.—A characteristic growth of the typhoid bacillus when planted on sterilized potato; it is almost invisible and threads are formed which have a beautiful serpentine motion. The potato is generally slightly acid and this is favorable for a typical growth.

GELATIN PLATE CULTURE.—The surface colonies are at first small and yellowish, punctiformed, becoming round and slightly notched and shining. The periphery is clear and transparent, slightly gray; the centers are opaque and slightly elevated. When magnified about seventy-five times young colonies are colorless and smooth bordered; later lines are visible like bands extending from the center; these gradually deepen like folds and look like hen tracks. The colony has a golden yellow color and is very pretty. The deep colonies are whetstone in shape and yellow, with smooth borders and slightly gray.

AGAR CULTURE.—The surface colonies are irregularly round, smooth bordered, gray and shining, and when magnified appears bright yellow in color, becoming darker towards the center with transparent edges. Dark irregular lines run out from the center and give the colony a beautiful appearance. The deep colonies are yellow and finely granular; they are opaque, whetstone shaped, looking something like an almond. The agar streak is even bordered, slightly elevated and slightly gray, with a lustrous appearance. After a time the color changes to yellow.

The study of this bacillus in cultures is most interesting and instructive and if care is exercised there need be no fear in cultivating and handling it. There are so many points of interest connected with its life, its manner of growth and its behavior under certain conditions, that the study of its biological characteristics will enable the student to intelligently investigate any other organism.

Typhoid bacilli produce a poison which interferes with their reproductive power, so that cultures will cease growing after a time and the bacilli will not be as actively motile as when planted in fresh nutrient media. When growing in the human body the poison is carried away in the blood and it is possible to determine quite early if the patient is attacked by the fever. The test is made with the serum from the blood and is called the agglutination test, which may be described as follows: A homogeneous suspension of the bacilli is made first. This is done by taking a platinum loop full of the germs from a culture about one day old, which has grown on hard agar; the germs are loosened from one another by rubbing them against the side of a test tube containing I c.c. of bouillon. When there is a perfect separation and even distribution the mixture is termed a homogeneous suspension, and a small quantity must be examined under the microscope with a magnification of 500 diameters to make sure that there are no clumps, and that all the bacilli are actively motile. This being ascertained, different dilutions are made of the blood serum, and each dilution is inoculated with typhoid bacilli. This is done on a cover-glass which is then inverted over a cell in a hollow-ground slide and sealed to avoid evaporation. The hanging drop is then placed in the incubator for various lengths of time, and occasionally removed and examined under the microscope to ascertain if the bacilli have gathered in bunches. If the blood has been taken from a typhoid patient, the agglutination is almost sure to take place even in weak dilutions. A positive aggultination is fairly conclusive evidence of enteric fever. See Plate 62.

Persons who have had typhoid fever remain immune for a considerable time, and in some cases for life. Blood from such

persons sometimes gives a similar reaction as the blood from a typhoid fever patient, but not quite so marked, especially in the great dilutions.

The examination of water to determine the presence of typhoid is quite difficult, because there is a class of bacteria called Coli commune (of which there are a number of species), which closely resemble the true typhoid bacilli. These species are found in the faeces of healthy persons and water is usually condemned as unfit for drinking purposes, when any of these are found, because it indicates that it is contaminated with sewage.

The biological characteristics of the typhoid bacillus may be thus summed up: It is aerobic and facultative anaerobic, clouds bouillon, does not coagulate milk, and the reaction is amphoteric, does not form spores, is not chromogenic, produces sulphuretted hydrogen in abundance, ordinarily does not produce indol, produces some acid in grape-sugar-bouillon, produces no gas in sugar agar and grows fairly well in an atmosphere of carbonic acid gas. It produces a ptomaine which is a powerful poison. The study of this organism is most interesting to the food chemist and investigator.

Typhoid is commonly epidemic, and it is no doubt carried from one location to another in food and water, which gives it a place in the catalog of dangerous bacteria associated with food spoilage.

CHOLERA BACILLUS.

The germ which produces Asiatic cholera is called by the following names: Comma Bacillus, Vibrio cholera, Spirillum cholera and "Bacille vir gule" (French). The name comma bacillus was given to it on account of the bodies often seen at the end of the germs, which are incorrectly described by Huppe as arthrospores. The germination of these bodies has not been positively observed, but they cause the germ to look like a comma (,) hence the name.

It is seldom that this terrible foe of man gets a strong hold in America, yet there have been some severe epidemics which carried whole families and communities out of existence in a few days. In various parts of Asia it is epidemic nearly all the time. It is said that in Saigon, Asia, there are always a few cases, and at times it spreads, carrying death to thousands.

Not until 1884 was it known positively just what was the agent of this dreaded disease. Dr. Koch of Berlin made the discovery of the germ and showed how it was carried in water and in food from one place to another. Dr. Koch carried on his researches in Egypt and India, and other eminent bacteriologists, both from Europe and America, have made searching investigations of the micro-organism and have studied its biological characteristics to

such an extent that the disease is now combated with more success than formerly when the true cause was shrouded in mystery.

The disease makes its appearance in every country occasionally, the specific organism being carried from infected districts by travelers, in articles of food and clothing, etc. The organism is one of the few which produces several ptomaines and toxic poisons, some of which are very dangerous. The specific action of these poisons are varied, and violent sickness and even death may result from them if taken in food which has been contaminated. Of course there is danger only when the disease is rife in certain localities, and when food stuff is obtained from such places.

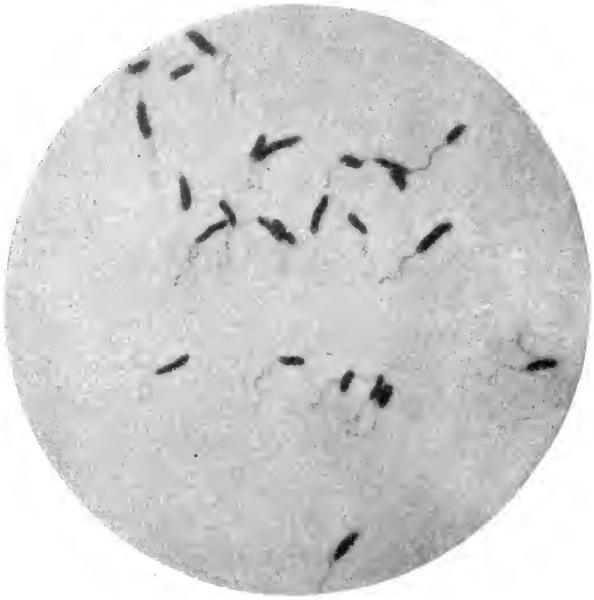


Plate 63

The Cholera spirillum is a curved rod measuring from 0.8 to 2μ in length and 0.3 to 0.4μ broad, the ends being rarely in the same plane, so that when several are united they resemble a corkscrew. When seen singly they resemble a comma and when seen in pairs may form the letter (S) or the letter (O). The (S) forms are quite common where the growth is rapid. The long corkscrew forms are seen generally in hanging drop cultures growing in favorable temperatures or conditions. Old cultures assume varied forms, bearing little resemblance to young cultures. Involution forms

with spherical bodies are produced, which are thought to be spores belonging to the so-called anthrospore type of spore formation, but no one has observed the germination of these bodies. It is a motile organism having a single terminal flagellum; sometimes two flagella.

Milk is a good medium for growth and is coagulated with the production of lactic acid.

Indol is formed abundantly in nutrient media containing peptone or albumen. The presence of indol in cultures may be demonstrated by adding a minute quantity of muriatic or sulphuric acid to the medium, when a rose-red color will make its appearance and is known as the nitrose indol reaction. In all cultures containing albumen indol is formed first and then the nitrates are converted into nitrites. This is also true of at least two other species of bacteria. All cultures of the cholera have a disagreeable, sickening odor, but these are not specially characteristic.

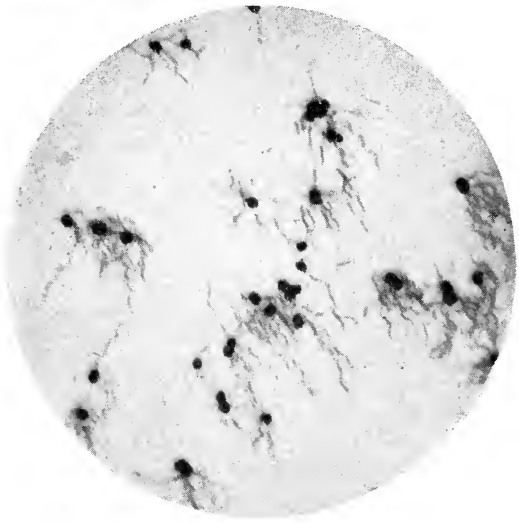


Plate 64. Bacillus Coli Communis, showing Flagella

Stained by the author. Magnified 1,000 diameters.

Media containing either milk-grape or cane sugar favor the production of lactic acid, which is dextrorotatory.

A beautiful chemical reaction is seen when cholera is grown in a test tube containing litmus milk; a pale blue pellicle is formed on the top, under which is a stratum of red, while the lower portion will be entirely decolorized.

Sulphuretted hydrogen (H_2S) is formed abundantly in peptone bouillon and also in sterilized egg albumen. Cholera germs are not very resistant to unfavorable conditions. They are killed in water heated to 130 degrees F., and when frozen they die within a

Bacillus Coli Communis, Escherich (1885)

BACTERIUM COLI COMMUNE; THE COLON BACILLUS; EMMERICH'S BACILLUS; B. NEAPOLITANUS.

Origin.—Found in the intestinal contents of man and animals, especially in the colon; also occurs in the discharges of healthy infants and in summer diarrhoea. It frequently accompanies the Comma Bacillus in the discharges of Asiatic cholera. It resembles in many respects the typhoid fever bacillus.

Form.—Short, narrow rods, varying in length from coccus-like forms to rods four to six times as long as wide. Usually found in pairs, may form threads.

Motility.—Depends upon the medium, age and temperature. It has diffuse flagella and giant whips.

Sporulation.—Has not been observed.

Anilin Dyes.—Stain readily. Gram's method will not stain. Bi-polar stain frequently occurs; also plasmolytic changes, as in potato cultures.

Growth.—More rapid than that of typhoid bacillus.

Plates.—On gelatin plates dull-white surface colonies, with irregular border and markings in the outer zone, are formed. These colonies are flat, spreading and aniso-diametric. The deep colonies are round or oval in form and of a yellowish color; they are frequently divided, forming lobulated masses. The round colonies have usually a yellow granular center surrounded by a colorless homogeneous ring. Does not liquefy. Strong odor of indol and amine. Owing to the ammoniacal reaction, the gelatin deposits a cloudy precipitate between the colonies and along any scratches that may occur on the glass plate. These characteristics differentiate it from the typhoid bacillus.

Stab culture.—Along the line of inoculation the growth is rather energetic. On the surface a white film with wavy border is formed.

Streak Culture.—On agar, a moist, white, spreading growth is formed; old cultures sometimes show needle-shaped crystals. On potato, an abundant, yellowish, moist slowly spreading growth is formed.

Milk.—Coagulates in one or two days; sometimes a week or more may be required.

Bouillon becomes very cloudy, with heavy sediment; at the surface a thick ring may adhere to the glass; a broken pellicle may form. Marked indol reaction.

Oxygen Requirements.—It is a facultative anaerobe.

Temperature.—Grows best at about 37° C., but will grow well at ordinary temperature.

Behavior to Gelatin.—Gelatin is not liquefied.

Aerogenesis.—When glucose is present carbonic acid and hydrogen are produced abundantly. Acid and gas may be formed in lactose media —unlike the typhoid bacillus.

Pathogenesis.—Guinea-pigs are very susceptible; rabbits less susceptible, and mice are insusceptible. Diarrhoea, collapse and death are produced in one to three days by small intravenous injections or injections into the abdominal cavity. The small intestine is hyperemic, more or less intensely inflamed; serous exudates may be present. The bacilli are abundant in the blood, in organs and on the peritoneum. Subcutaneous injections produce only a local abscess usually; is not usually fatal.

few days. Drying kills them in a few hours. Weak antiseptics destroy them. They require frequent transplanting in favorable nutrient media to keep them for any long period.

The bacterial poisons formed by the cholera germ are very powerful. Old bouillon cultures, when filtered through the Chamberland filter has all germs removed, but the poison in solution kills small animals, such as rabbits, mice, guinea pigs, etc.

Putrescin and cadaverin are two ptomaines extracted by Brieger from cholera, but are not very poisonous. Methyl-guanidin is another, but is very poisonous, causing muscular tremors and death. "Toxopepton" was obtained by Petri as a poisonous proteid which killed guinea pigs in a few hours in doses of 36 grams per kilogram weight.

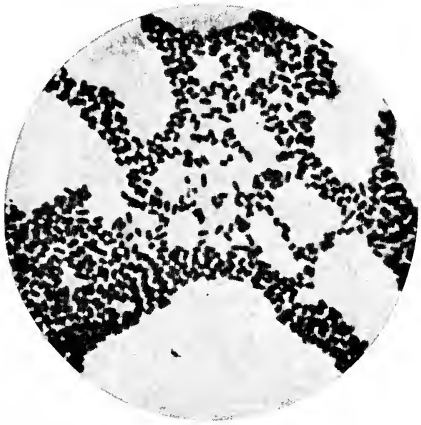


Plate 65. Bacillus Coli Communis

Magnified 1,000 diameters.

A substance insoluble in water and acids, but soluble in alkalis and ether is obtained by a method discovered by Winter and Lesage. When evaporated from the extract, it is oily and becomes yellow like fat on cooling. Small doses are fatal in a short time when fed to small animals.

BACILLUS COLI COMMUNIS OR COLON BACILLUS.

This very common bacillus is not a single species, but a family comprising a number of species very closely resembling one another and differentiated only by most careful study. It is a question whether there are distinct species or whether the one species changes in its morphological and biological character under certain conditions, thus causing confusion. Various textbooks attempt a separation of species according to their different pathogenic power, but

in my own researches I have been unable to make a clear distinction between any germs of this family. The Coli communis is always present in intestines of healthy man and various animals, and is nearly always found associated with other bacteria in a number of diseases, such as suppurative processes in the internal organs, infectious enteritis, ulcerated liver, puerperal fever, meningitis, etc.

The bacillus resembles the typhoid bacillus so closely in its form, size and its growth on various culture media as to make its differentiation a nice bacteriological problem.

The differences may be stated as follows: It is not so actively motile as typhoid, has fewer and shorter flagella, it develops more luxuriantly on all media, and the growth on potato is visible, while that of typhoid is not. It coagulates milk, giving marked acid reaction, while typhoid does not and the acid reaction is only slight. It forms considerable gas in media containing glucose, lactose and saccharose. Colonies are pink on (alkaline) agar and gelatine media containing lactose and litmus tincture, while those of Typhoid are pale blue.

It produces indol (in peptone solutions) more freely than typhoid. Typhoid as a rule does not produce indol, but it is sometimes formed in peptone solutions.

Agglutination test is generally negative. Pfeiffer's serum reaction with typhoid blood serum as described under typhoid is negative with the Coli communis.

Tyrotoxicon is supposed to be a poison formed by this microbe, although the evidence is not positive. In 1886 this poison was first discovered in milk which had poisoned a number of persons at Long Branch. The milk had been exposed to unusual conditions, favoring the growth of the Colon bacillus probably. Vaughan reported an interesting case of poison from tyrotoxicon, known as the Milan case. The symptoms were rapid pulse, breathing rapid, burning sensation in the throat and stomach, abdomen retracted and severe throbbing in the abdomen. The contamination of milk from germs which were present in decomposing matter under the floor was found to be the cause. Vaughan and Novy found the poison in numerous samples of poisonous ice cream and custard. Novy says that "Undoubtedly there are many forms of the Colon bacillus which frequently find their way into milk and, on account of the toxins contained within their cells, they render this and various other foods, of which milk is a constituent, more or less poisonous." The potassium compound of tyrotoxicon is not decomposed in a temperature under 265 degrees F., so that foods containing milk and cheese which have been exposed in any way to human or animal excreta or to contaminated water are liable to have this poison formed by the Bacillus coli communis. The poison is present in the human

faeces, but ordinarily passes away without any serious results, as it is formed past the danger line.

Tyrotoxicon may be formed by other agents, but the accurate knowledge of its origin is not yet thoroughly investigated. It is a powerful poison and many fatalities have followed where food containing it has been eaten. It is generally found in milk, ice cream and cheese, from which it derives its name.

TETANUS.

Tetanus or lockjaw is a disease produced in man and nearly all domestic animals by a widespread germ found in garden soil, manure heaps and saltpeter beds. The horse is the most susceptible domestic animal, but cows, sheep, pigs and goats are sometimes attacked. Man is very susceptible to this disease, which is considered to be one of the most dangerous and deadly.

The germ produces several very powerful ptomaines, which have been isolated by Brieger in crystalline forms; *Tetanine,* which decomposes in acid and remains unaltered in alkaline solutions, will in itself produce lockjaw when injected into the tissues of animals; *tetanoxin,* which produces tremors and paralysis, followed by convulsions; *tetanotoxin,* which induces lockjaw, accompanied by a flow of tears and saliva; and *spasmotoxin,* which produces spasms and convulsions. Late investigators have formed the opinion that the crystalline substances obtained by Brieger owed their poisonous properties to the toxin of tetanus and that they were not of themselves poisons.

The flesh of animals suffering from disease is apt to contain such elements, and if such flesh should be used in the preparation of food products, either in soups, extracts or canned meats, the direful results may be far reaching. Packers who use meats can thus understand how necessary it is to be careful in their selection; any unnatural appearances should be a sufficient reason for rejecting any lot of meat which they may be using.

The poison of the tetanus germ has been used by natives of the New Hebrides, according to Ledantec, on arrowheads made of human bones, which they first cover with resin and smear with the slime found in swamps; the slime no doubt contains the tetanus bacilli in large numbers and even a slight wound from such weapons of warfare would prove fatal.

The tetanus bacillus when magnified one thousand diameters is seen to be slender, with a large spore in the end of the rod which does not readily take a stain. In measures from 3 to 5 μ in length and 0.3 to 0.5 μ in width. In cultures it is seen to grow in threads and often appears without the spore, but usually the spore is present in single rods and gives the germ the appearance of a nail, from

Bacillus Tetani, Nicolaier (1884)

TETANUS, LOCK-JAW; WUNDSTARRKRAMPF (GERM.); TETANOS. (FR.)

Origin.—It is found in animals that die of tetanus after inoculation with earth; in traumatic tetanus of man and animals; in head tetanus; tetanus of new-born; is present in the intestines.

Form.—Large, narrow rods, having rounded ends; may form threads.

Motility.—It is motile. Many curly flagella, also giant whips.

Sporulation.—Spores are formed rapidly at 37°. Terminal spores are formed, with enlargement-drum-sticks.

Anilin Dyes.—Stain readily. Gram's method may be used.

Growth.—Slow.

Plates.—Colonies develop in gelatin at ordinary temperature in four to seven days; these resemble those of the Hay bacillus. The gelatin slowly liquefies, and gas is produced. On agar plates the colonies have the appearance of faint clouds; when examined under the microscope these are seen to be oval, partially surrounded by a whorl of extremely fine threads.

Stab Culture.—No growth at the upper part of the tube. In glucose gelatin tubes cultures show a cloudy growth along the line of inoculation, which radiates outward into the gelatin, resembling that of the Root bacillus. The gelatin is eventually liquefied. Gas bubbles are present. In glucose agar at 37° the growth is sometimes indistinct, showing radiations.

Streak Culture.—On glucose agar growth is rapid and practically invisible.

Bouillon.—It becomes diffusely cloudy at 37°, but after several days the growth settles to the bottom in the form of a scarcely visible sediment.

Glucose Gelatin, colored with litmus.—Becomes permanently liquefied at 37°, a very small sediment is formed. The culture remains blue, thus showing that there is no acid formation.

Milk.—Grows well in milk, but produces no change. Starch is not inverted. On potato, the growth is invisible.

Oxygen Requirements.—It is an obligative anaerobe, growing in vacuum, hydrogen, carbonic acid and nitrogen.

Temperature.—Grows best at about 38° C. Will not grow below 16° C.

Behavior to Gelatin.—Liquefies.

Aerogenesis.—Produces gas; has disagreeable odor; H^2S.

Attenuation.—There is a loss of virulence by culture.

Immunity.—Iodin trichlorid; thymus bouillon cultures; injection of filtered cultures; of purified toxin; milk of immunized goat; blood-serum of rabbits, dogs, sheep, horses, which have been artificially immunized. The tetanus toxin is destroyed by the nucleohiston from the thymus gland.

Pathogenesis.—Man, horse, sheep, young cattle, goats, guinea-pigs, white rats and white mice, are susceptible. Dogs and rabbits are less susceptible. Chickens and ducks, immune. It is not present in the blood, but occurs in small numbers at the point of inoculation; may be absent entirely at times. Products are intensely poisonous. A guinea-pig may be killed by 0.002 c. c. of a filtered bouillon culture and a dose of 0.0002 c. c. may kill a mouse. Disease cannot be produced by pure tetanus spores. Mixed infection.

Infection.—Occurs through wounds. The poisoned arrows of the New Hebrides contain tetanus and malignant edema spores.

Diagnosis.—On account of its scarcity and the presence of other aerobic and anaerobic bacteria, the bacillus is hard to detect. The pus should be taken from the wound by means of a sterile drawn-out glass tube pipette and transferred to glucose litmus gelatin. A loopful of this dilution should then be transferred to each of eight or ten tubes of liquefied glucose agar. These should be poured into Petri dishes and developed in hydrogen. The characteristic colony is oval, surrounded on one end by a whorl of threads.

The original glucose litmus gelatin is developed at 35°; a portion of this is injected under the skin of a white mouse or a guinea-pig.

A portion of the pus should be stained direct, then examined for tetanus bacilli and for the terminal spores (drum-sticks). (From Novy.)

Plate 66 Bacillus Tetanus Flagellated

Magnified 1,200 diameters.

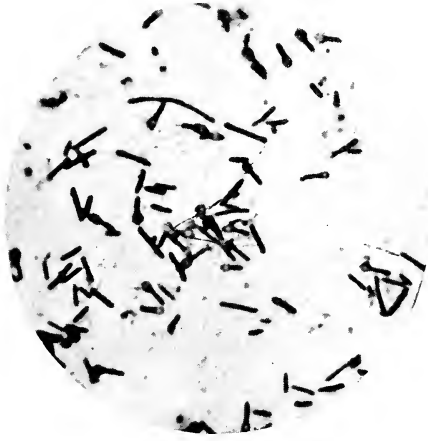


Plate 67. Bacillus Tetanus

Magnified X 1,000. Photomicrogrash from Slide Prepared by Author from Bouillon. Stained with Carbol Fuchsin. Large Terminal Spares are shown, which give to the Bacilli the Appearance of Nails or Pins. It is sometimes called the "Nail Head Germ."

whence it received the name of "the nail-head germ." The spore is much greater in diameter than the width of the bacillus and gives it the appearance of a pin or a clove or an eyescrew. Spores may be seen in the long threads at times.

The spores remain alive and virulent in culture media for more than a year (in a dark place), but are not able to live through a boiling temperature of 212 degrees F., applied for ten minutes. Five per cent. phenol solution kills them in one day, but if muriatic acid is added the spores perish within a couple of hours. The vitality may be tested by transplanting after exposures to heat and antiseptics. Bichloride of mercury 1-1000 destroys them within three hours, but when muriatic acid is added to the mercury they perish in less than an hour.

The young bacilli may be stained for flagella and the number is surprisingly large and it is difficult to count them. Votteler claims to have counted 50 to 100 growing out from the entire surface. The bacilli are not actively, motile which is more surprising from the fact that other germs with one-fifth the number of flagella are very active. There is, however, a distinct spontaneous motion which may be seen in the hanging drop cultures.

Tetanus bacillus is strongly anaerobic, even small quantities of oxygen interfere with its growth, especially if it is cultivated from the animal body or wounds which have become infected. After transplanting a number of times in nutrient media the tetanus germs become less susceptible to oxygen and have been cultivated quite successfully in the presence of other organisms belonging to the class of aerobes. When grown as an aerobe, pure cultures lose much of their virulence. This is also true of other anaerobic bacteria belonging to the pathogenic classification.

Bacillus tetanus grows well at 98 to 100 degrees F. and slowly at room temperature. At 50 degrees F. there is no growth. When the germs are very virulent there is only a moderate growth, even at 98 degrees F., but cultures that have been frequently transplanted grow most luxuriantly. Many pathogenic bacteria seem to change in their nature and develop the characteristics of the putrefactive species and lose much of their virulence after repeated transplanting. These germs when inoculated into susceptible animals become highly virulent again.

The cultivation of tetanus in artificial media is made regular anaerobic culture apparatus by using hydrogen as an atmosphere after driving out all oxygen, but the least expensive method is the absorption of oxygen by pyrogalic acid in the presence of potassium hydroxid, enough to make the acid alkaline, when it will darken, first brown and then black.

Animals which have tetanus will have the active poison throughout the circulating system. The blood and gland secretions contain it in sufficient quantities to set up the disease in other animals which are inoculated with the blood or pleural exudes. Strange to say, the tissue of diseased animals seems to have to be devoid of the poison, except at the point where the germs had gained entrance i e., the wound or seat of inoculation. The poison becomes inert if subjected to temperatures varying from 150 to 212 F., but if it is dried at ordinary temperature first it retains its virulence and withstands much higher temperatures.

When the germs gain entrance into a living animal or man through a wound the point of entrance will heal over and appear to be getting well. This places the tetanus bacilli in an anaerobic and they soon begin to multiply, the period of incubation being from one to twenty-five days. If lockjaw results after one day there are probably a number of pus germs in the wound, which use up the oxygen from the surface and place the tetanus bacilli in their favorable anaerobic condition, in which case the sufferer has small chance of recovery. The longer the period of incubation the greater is the chance for recovery, because the system becomes more or less armed against the poison by gradually accommodating itself to its influence.

The anti-tetanic serum is prepared from the blood of inoculated animals and has been injected into the blood of victims of tetanus with satisfactory results. The serum is known to produce immunity, and hundreds of positive cures are recorded from its use.

There is no doubt but that the flesh of animals suffering with lockjaw contains large quantities of the toxin in the juices, and if used as food either in manufacture of canned meats or soup stock, will cause severe sickness and possibly death. It is certainly a dangerous malady, from the fact that an animal may have the disease during the period of incubation and show no outward sign of its presence. There is always an exceedingly abundant production of H_2S (sulphuretted hydrogen) where the germs are growing and indol is produced, so that infected food products will show signs of contamination if care is exercised in inspection.

BACILLUS DIPHTHERIAE COLUMBARUM.

This is a non-motile organism, about 2μ in length and 0.5μ broad, having rounded ends. There is no spore formation, and it is aerobic and does not liquefy gelatin. The flesh of chickens suffering with this disease presents a flabby appearance and is usually somewhat discolored. Chickens, game birds and pigeons are susceptile, and it is very contagious among them.

There are numerous cases of ptomaine poisoning on record due to bacteria which have produced the poison in chicken; indeed, this

is quite common. One case happened three years ago in Aspinwall, Pa., at a chicken and waffle supper. Fully twenty persons were made violently ill from eating the chicken. One case of poisoning from chicken soup came under my notice during a visit to San Francisco. In this case the soup had been canned and was perfectly sterilized, yet the ptomaine remained. Just what organism caused the trouble I do not know, because I was not in a position to make a thorough examination, but part of the soup when fed to a dog caused the animal to become violently sick. There is no doubt but that Bacillus Diptheriae Columbarum is one organism which produces a powerful toxic poison and that chickens suffering with diptheria have the poison in sufficient quantities to produce severe cramps and nervous prostration with symptoms of paralysis in persons who partake of the cooked flesh. There is no doubt that such diseased poultry is sometimes sold on the market, and packers of potted chicken, chicken soup and canned chicken should be extremely careful in selection. If careful inspection is made, evidence of disease will almost invariably be indicated by general appearance and color.

Bacillus Diptheria Vitulorum is another organism affecting calves and is found in the mouth, lungs and intestines. It is usually in filaments, and is several times as long as it measures in breadth. It is non-liquefying on calf blood serum, which seems to be the only medium on which a culture can be obtained.

The flesh of a calf suffering from diptheria is not sound in color, nor is it firm, and the usual precautions will suffice to aid the inspector in judging it.

There are a number of diseases affecting animals and poultry which are due to pathogenic bacteria, but I will merely mention a few of them, as our readers are pretty familiar with the subject by this time. Vibrio Metschnikovii, which is the organism producing one kind of chicken cholera. Bacillus of Chicken Cholera, Hog Cholera Bacillus, Anthrax Bacillus, and various organisms which produce blood poisoning.

It must not be supposed by our readers that meats and albuminous food materials, which contain poisons due to the organisms we have been describing, are constantly put on the market and sold to unsuspecting packers. It happens only rarely that such material is used, and our object has been to furnish complete information on this subject to teach packers the necessity of careful inspection, both macroscopically and microscopically. Ptomaine poisonig from canned goods is extremely rare in comparison with such complications from other sources, but we hope to decrease these by fully describing the various organisms known to produce poisons and by pointing out the danger in using unsound material.

Every packer should take a lively interest in this subject, because cases of Ptomaine poisoning, although rare, are a source of considerable annoyance to the whole industry. Unreasonable bills are introduced in the legislatures of various states which cause the packers trouble. I have referred in former pages to the "Canned Goods Dating Bills."

PTOMAINES AND TOXINS.

Ptomaine Poisoning and Its Bearing on the Food Industry.

The word ptomaine springs from the Greek word (*ptoma*) ptoma, which is pronounced (toma) and means a dead body. This name was given to a group of poisons obtained from cadavers by Selmi, a noted Italian chemist and toxicoligist. For many years the term was applied to all bases which combined with acids and formed salts as a result of bacterial activity, and these bases were regarded as poisons, but later investigations have demonstrated that ptomaines are not all poisons, in fact only a few of them may be considered as dangerous. Many of the ptomaines owe their poisonous properties to a more powerful poison, which is termed a toxin, and this is a product of the putrefactive and pathogenic bacteria, many of which we have described. Novy describes a ptomaine as, "an organic chemical compound, basic in character, and formed by the action of bacteria on nitrogenous matter. On account of their basic properties, in which they resemble the vegetable alkaloids, ptomaines may be called putrefactive alkaloids."

They are formed in the putrefactive processes on both vegetable and animal matter of albuminous nature by the agency of bacteria. When such matter is attacked by bacteria, the various molecules containing carbon, nitrogen, oxygen and hydrogen, are upset and new atomical relations are formed by the cleavage, in the various steps of total dissolution. The final results of bacterial activity is the fomation of carbon dioxid, ammonia, and water, and it is between the first and last stages that the alkaloids are formed.

In some cases of food poisonings, toxins have been isolated as well as ptomaines, so we will include both in the discussion of this subject.

The increase of food poisoning in recent years has been declared by some eminent authorities to the increased consumption of preserved foods, the claim being made that inferior stuff, which would not be purchased in the raw state on account of its appearance and partial decomposition is easily made to look well by skillful chefs and manufacturers of canned foods, and that these contain the poisons elaborated by harmful bacteria, and that these poisonous alkaloids do not lose their potency in the cooking and sterilizing

processes. Canners have been accused of using partially decomposed materials, both knowingly and ignorantly, and the responsibility for a large number of food poisoning cases, has been charged against them. For the most part ignorance has been charged because they are not familiar with the scientific principles of their business, and do not realize the dangers lurking in decomposing material, due to vital activity of bacteria.

While there may be extremely little truth in these statements and while we must admit that the canners and preservers of food products have been guilty of gross ignorance, and are not even today well informed on these matters, we cannot help making the statement that the charges are very much overdrawn and that the ignorance displayed by the canners could hardly surpass that displayed by the list of physicians, who are for the most part to blame for the charges of ptomaine poisoning against canned goods. In order to write intelligently on this subject, I have made it a point to question a number of physicians concerning ptomaines and toxic poisons, and their answers showed that they were as a rule ignorant of their names and origin.

I do not mean to cast reflections upon physicians in general; many of them are conscientious in their diagnoses and would not place the blame on canned goods without investigating the cause carefully. There are others, however, who jump at conclusions and furnish the press with information which may be absolutely untrue. In this manner false statements have been circulated throughout the civilized world and the preserving industry has had to bear the brunt in many cases.

It is not true that canners and preservers are in the habit of using partially decomposed material. It is impossible to make fine goods from anything but the very best raw material, and all reputable firms are extremely careful in selecting the very best, and their contracts with farmers are strict.

We must admit, however, that there are some packers who are ignorant of the dangers of which we are writing and there may be some who are unscrupulous, but the goods turned out by these manufacturers are very inferior, often highly colored to cover up imperfections, and the quality is very poor. There have been, and probably are today, a few packers who come under these two classes. *A good Natural Pure Food Law* will be a great blessing to the whole industry, and will eliminate all goods artificially colored, and unnecessarily preserved with antiseptics. When this law is made effective, the honest and well informed food preservers will enjoy a ready market for all their goods, and will not be embarrassed by persecutions.

When all, or nearly all, goods of inferior quality are eliminated, there will be renewed confidence in manufactured food products, and the worry of putting up goods at home will form a part of past history. The superiority of manufactured food products over home made goods is conceded by the intelligent consumers, for the reason that only men of great skill are employed to produce goods of fine quality, and by the use of improved machinery the *little* imperfections seen in the home goods are overcome.

The progress now being made in the science of bacteriology, and the research work in physiological chemistry, are furnishing considerable literary material, which is being published to enlighten manufacturers on these subjects. The problems of spoilage are now taken up carefully, and the various ptomaines and toxins are becoming known, also the bacteria which are responsible.

The isolation of ptomaines and toxins requires great skill and patience, and there are only a very few men who are far enough advanced to make these analyses, and our readers will be able to judge of this from the examples we will give as illustrations.

We will not attempt in a work of this kind to give a complete list of all these poisons (and methods of extracting them), but if anyone desires to enter into the study in a comprehensive manner we will be glad to furnish material for such research. Manufacturers who have suits brought against them on account of reported ptomaine poison found in their goods need some direct information on the methods employed by chemists in isolating these poisons, in order to supply their lawyers with the necessary questions to be asked the physicians who testify in such cases. The methods described here will show how complicated the analyses are and it is safe to say that they will be sufficiently advanced to enable a good attorney to overthrow mere guesswork of many physicians who testify in these cases. Not long ago a certain well-known firm was called upon to make defense in suit for damages; the parties claiming ptomaine poisoning where canned goods were used at a meal, after which one member of the family was taken violently ill. Three doctors were called in, and worked all night to save the woman, and succeeded, but the family being poor, and the doctor's bills large, a suit for damages seemed to be a good way to even up. When the case came up, and the attorney, (armed with the chemical methods for extracting ptomaines) asked the doctors the various questions, the case seemed ridiculous and was thrown out of court. In July, 1903, when the writer was in St. Paul attending the Convention of State Food Commissioners and Chemists, an article appeared in the papers stating that a family had been poisoned with food containing a ptomaine, and the physician who attended the patients was quoted as authority for the statement. Samples of the

food and some of stomach contents were sent to the bacteriologist of St. Paul and the analysis proved it to be a common mineral poison and not a ptomaine. The cooked beefsteak was found to contain this poison. How the poison happened to be there no one was able to learn, but the point I wish to make is that ptomaine poisons are not always responsible.

These cases demonstrate the hasty conclusions often reached by doctors. To be sure they furnish sensational reading, but the manufacturer of foodstuffs is put to considerable worry and expense to defend himself when suits are entered against him for damages.

In making examinations of suspected food, the samples should be brought to the laboratory without delay to lessen the change of other germs gaining entrance. The germs present on the inside of the material are probably the cause, and cultures are prepared according to the well-known plate methods. Some of the plates are incubated in the anærobic, and others in the ordinary way, so that all the bacteria present may come under the eye of the analyst. There is hardly ever enough of the suspected food to make a chemical test for a ptomaine or toxin, because it is impossible to extract the minute quantities of these powerful poisons except from large quantities of material. If any appreciable amount of poison of bacterial origin could be isolated from a small quantity of material, it would be so powerful as to kill in a short time all the affected persons. One gram of tetanotoxin is calculated to be sufficient to kill 4,500 people.

After the germs have been isolated, those of poisonous character are easily identified, and each species is then tested on such animals as mice, rats, kittens, puppies, rabbits and guinea-pigs by feeding, by subcutaneous, inoculation, by ultra-peritoneal inoculation and by intravenous inoculation. Various animals are so treated, because some may not be susceptible. The inoculating fluid is generally a bouillon culture of the germs, twenty-four hours old, and the amount used is from one to ten cubic centimeters. Sometimes the inoculation is made with the filtrate of a bouillon culture, from which all living germs are held back by filtering through porcelain. After making these tests the analysis for the ptomaines and toxins are conducted as follows:

Only absolutely pure chemicals can successfully be employed in this work and this must be ascertained beforehand by evaporating the ether used and analyzing the residue for poisonous bodies.

The Stas-Otto Method. (Vaughan and Novy.).

This method depends upon the following facts: (1) The salts of the alkaloids are soluble in water and alcohol and generally insoluble in ether, and (2) the free alkaloids are soluble in ether and

are removed from alkaline fluids by agitation with ether. These principles are capable of great variations in their application. The usual directions are as follows: Treat the mass under examination with about twice its weight of 90 per cent alcohol, and from ten to thirty grains of tartaric or oxalic acid; digest the whole for some time at about 158°F. and filter. Evaporate the filtrate at a temperature not exceeding 95° F., either in a strong current of air or in vacuo over sulphuric acid. Take up the residue with absolute alcohol, filter, and again evaporate at a low temperature. Dissolve this residue in water, render alkaline with sodium carbonate, and agitate with ether. After separation remove ether with a pipette, or by means of a separator, and allow it to evaporate spontaneously. The residue may be further purified by redissolving in water and again extracting with ether. Chloroform, amylic alcohol and benzene are used as solvents after extraction with ether.

Brieger's Method.—The substance under examination is divided as fine as possible, and then heated with water slightly acidified with hydrochloric acid. During the heating care must be taken that the feebly acid reaction is maintained and the heat should continue for only a few minutes. The liquid is then filtered and concentrated, at first on a plate, and then on the water-bath, to a syrup. An extraction of the syrup is made with 96 per cent. alcohol and the filtered extract is treated with a warm alcoholic solution of lead acetate. The lead precipitate is removed by filtration, the filtrate evaporated to a syrup and again extracted with 96 per cent alcohol. The alcohol is driven off; the residue taken up with water; traces of lead removed with hydrogen sulphid; and the filtrate acidified with hydrochloric acid, evaporated to a syrup, which is extracted with alcohol, and the filtrate precipitated with an alcoholic solution of mercuric chlorid. The mercury precipitate is boiled with water, and on account of the differences in solubility of the double compounds with mercury, one ptomaine may be separated from others at this stage of the process.

The mercury filtrate is freed from mercury, evaporated, and the excess of hydrochloric acid carefully neutralized with soda (the reaction is kept feebly acid); then it is again taken up with alcohol to free it from inorganic salts. The alcohol is evaporated, the residue taken up with water, the remaining traces of hydrochloric acid neutralized with soda, the whole acidified with nitric acid and treated with phosphomolybdic acid. The phosphomolybdate double compound is separated by filtration and decomposed with neutral acetate of lead. This is hastened by heating on the water bath. The lead is removed by hydrogen sulphid, the filtrate is evaporated to a syrup and taken up with alcohol, from which many ptomaines are deposited as chlorids, or double salts may be formed in the alcoholic solution.

The chlorids deposited from the alcoholic solution are seldom pure and may be isolated by precipitation with gold chlorid, platinum chlorid, or picric acid, and on account of the differences in solubility of these salts, the purification is rendered more easy. The chlorid of the base is obtained by removing the metal with hydrogen sulphid, while the picrate is taken up with water, acidified with hydrochloric acid, and repeatedly extracted with ether, in order to remove the picric acid.

These remarkable methods are not the only ones used in extracting bacterial poisons, but they are sufficiently complicated to test the skill of even first-class chemists and may be used as a means of defense against falsely reported discoveries of ptomaines in food products.

We will conclude this subject by naming some of the poisonous ptomaines which have been isolated by some of the most eminent chemists in the world. (Tetanotoxin $C_5H_{11}N$)—(Amylanim $C_5H_{13}N$)—Hexylamin $C_6H_{15}N$)—Trimethylenediamin $C_3H_8N_2$)—(Susotoxin $C_{10}H_{26}N_2$).—(Methyl guanidin $C_2H_7N_3$)—(Asellin $C_{25}H_{32}N_4$)—(Neurin $C_5H_{13}NO$),—(Cholin $C_5H_{15}NO_2$)$_6$(Mydatoxin $C_6H_{13}NO_2$)—(Mytilotoxin $C_5H_{15}NO_2$)—(Gadinin $C_7H_{17}NO_2$)—(Typhotoxin $C_7H_{17}NO_2$)—(Muscarin $C_5H_{15}NO_3$)—(Tetanin $C_{13}H_{30}N_2O_4$).—(Tyrotoxicon, Mydalein, Spasmotoxin, Adiamin, Peptotoxin and many others unnamed.)

CHAPTER VI.

Sterilization

Nature of Spores. Cleanliness in Manufacturing. Disposition of Waste Material. The Venting Process. Vacuum Machinery. Discontinuous Sterilization. Preservatives Formed in Sterilization.

STERILIZATION.

Its Application in Canning and Preserving

To sterilize any material is to make it barren, or to destroy the bacteria present, or render them incapable of reproduction. In its broad meaning, it might embrace the use of any chemical or physical force, capable of destroying reproductive powers, but in its restricted sense it means to apply heat to destroy micro-organisms or to hinder their vegetating power.

The large number of bacteria which do not produce spores are easily destroyed at temperatures ranging from 140 to 180 degrees F., moist heat, and to this class belong nearly all pathogenic, lactic and acetic bacteria, and also yeast and molds, although these are more resistant to heat than the bacteria mentioned. To the other class which produce spores belong the butyric, subtilis and mesenteric families, and a few to the Pathogenic. There are a great number of species belonging to these families, some of which produce spores of great vitality, and these spores are able to resist boiling for hours, owing to a thick membrane which protects the vital power within. They resemble dry beans, peas, corn, etc., and the older they are, the closer do their heat-resisting membranes enclose their life. I have seen beans so hard after several years drying, that they refused to absorb water for two weeks and then very slowly, so when spores become old it is reasonable to suppose that they shrink, and the pores of their membranes become impervious to moisture, so that they require very high temperature to deprive them of vitality. So small do these spores become, that even the microscope fails often to reveal their presence in fluids, and one writer has conceived the idea that they might not become wet, if air containing them should be forced through sulphuric acid. This might seem ridiculous on first thought, but when we consider their minuteness and the repellant force of certain substances against moisture, it does not seem so unreasonable. If we fill a

glass graduate with water, so that the surface is not disturbed, there will be a distinct difference between the height of the water at the center from that at the edge of the glass; the glass holds the rising fluid down below the service level, unless it be first moistened. A thin cover-glass will float on a fluid, although its specific gravity is much greater; the repellant force will bank up the fluid all around it, so that it will float below the surrounding surface of the fluid, which has no power to wet the upper surface of the glass. The same phenomenon may be observed if a small needle be gently laid on the surface of the fluid. To my mind it seems reasonable to suppose that some spores are so constructed that they will repel the surrounding fluid and do not become wet until certain changes are produced in that fluid, either by increased temperature or nutritive power, or by chemicals which may attack the cell membrane.

For general sterilizing purposes, the packers of hermetically sealed goods use moist heat. This kind of heat is far better than dry heat, because of the character of the cell membrane of bacteria and spores. It is by the absorption of moisture that bacteria are able to grow and vegetate, and their spores are enabled to send forth young rods. Steam heat therefore exerts a violent action against life and destroys it much quicker than the same temperature of dry heat. The action of the cell membrane against dry heat might be likened to that of asbestos. For sterilizing canned goods, the packers use several devices which are all good, but the thermal death point of the bacteria present in the cans is a problem.

There are a number of bacteria closely resembling one another, which produce spores greatly varying in heat-resisting power; the most resistant species known was discovered by Globig, which is found on potatoes, and is called the "Potato bacillus." This organism produces spores able to live through six to ten hours or more of boiling temperature. Almost all the bacteria having great heat-resisting power are found in cultivated soil, and are present on the stems, leaves and edible portions of all vegetables. If the juices of these plants become infested with spores of these various species, the problem of sterilization is a deep one; too much heat destroys the flavor of the canned product, and as a rule cannot be fixed broad enough to take in the thermal death point of all heat-resisting germs, since nearly all speices are able to grow on most of the vegetables used for canning, excepting tomatoes; for instance, the juice of peas may be used to cultivate in pure culture the "Potato bacillus," and we all know that a process sufficiently high to destroy the spores of this microbe would cook the peas to pieces and destroy the flavor. When decomposition of vegetables sets in, especially while yet in the field, or when they are piled up with the pods, leaves or

vines still present among them, the way is made easy for the development of numerous spore-bearing bacteria, which find a suitable nutritive material in the exposed juices, to multiply and produce spores. When cultures are made of the bacteria found on the stems, leaves, pods or husks, the varieties of spore-bearing bacteria found are numerous. As we have stated before, the most hardy bacteria are found in the soil, and they are growing rapidly on all dead matter which nature carries down to them; their spores are formed and under the warm summer sunshine they become dry and light, and are carried by currents of air to all parts of the field, finding lodgment on the growing plants, they lie dormant until they gain entrance to nutrient juices, when they begin a new life cycle and form the same hardy spores which are often found in cans of spoiled goods.

Now, to make the point clear, if packers use raw material which is bruised or partially decomposed, they have much to contend with, because they must process this material long enough to destroy all manner of bacteria, which gain entrance to the deep portions.

The value of a blanching bath for peas, string beans, asparagus, etc., is twofold; it arrests decomposition and it washes away many resistant forms of germs, but if the deepest tissues and fibres are penetrated, the washing does not carry away the destroying agents.

There are some points worthy of consideration here, and we refer to the nature of certain vegetables, and their nutritive value for various bacteria. Every kind of fruit and vegetable has a chemical composition peculiar to itself; some are strongly acid, while others are nearly neutral, some have considerable starch and albumen, while others are rich in sugar or carbohydrates; some have antiseptic compounds, such as benzoic acid, phenol, salicylic acid, creosote, formaldehyde, etc., but the amount is only small, yet sufficient to prevent the growth of many species of bacteria. There are certain species of bacteria which are always found associated with a given kind of vegetable or fruit, which furnish the germs with all their vital requirement; other species may be present, but are checked in their growth by the greater multiplication of the regular species. Certain bacteria peculiar to peas may be able to force entrance to the juices, while others would find nutrition only on exposed surfaces. This accounts for prevalence of certain species on a particular kind of vegetable.

The processor becomes familiar with a certain temperature, prolonged for a given time, which seems to sterilize the same vegetables, fruits, etc., year after year, without very much spoilage, but suddenly the old rule fails and whole batches of canned goods spoil because of a new species of bacteria having taken the field; the old process is not sufficient to destroy the spores of the new variety. How necessary it is to know the reason for this! Only a knowledge of bacteria can help him.

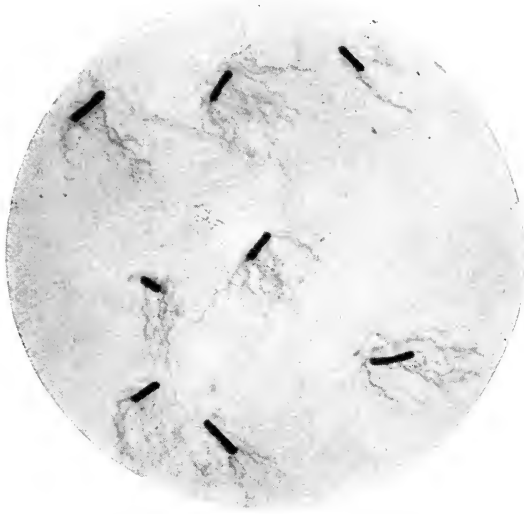


Plate 68. Globig's Potato Bacillus, Flagellated

Photomicrograph of Globig's Potato bacillus, showing numerous flagella. Magnified 1,200 diameters.

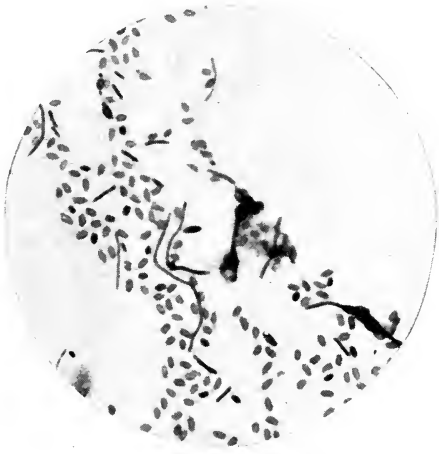


Plate 69. Globig's Potato Bacillus

Photomicrograph showiug Numerous Spores which are larger than the Rods in Diameter. Culture from Agar, isolated from Spoiled Corn. Stained with Fuchsin. Slide preparation by Author. Magnified X 1,000.

There are some natural causes for the presence of new varieties of bacteria which suddenly make their appearance in canned goods. The weather often has an influence on the chemical composition of raw material; during some seasons there may be more sugar and less starch, or there may be less sugar and increased acidity, due to rains or drought. We have all noticed that it is sometimes the very finest looking corn which spoils. We have seen tomatoes crack open by sunshine after rains, and later have heard the popping of the corks from the catsup bottles, or seen the cans swell in various piles.

The nature of the soil may have an influence. The farmers have been growing for us the same products year after year in ground cared for and manured regularly; suddenly it becomes necessary to procure a certain fertilizer and a number go in together and buy fertilizer which changes the chemical composition of the truck raised on the ground.

Now when changes take place in the chemical composition of raw material, it may not be just suited to the same varieties of bacteria formerly found associated with it, but the changed constituents form the nutritive elements necessary for the luxuriant growth of more formidable species.

The problem of sterilization becomes a scientific study, when such changes as we have mentioned take place in the composition of the raw material. To be sure, anyone may be able to sterilize any kind of product by giving it a high temperature for a long time, more than is actually necessary; but to know just how much is sufficient, and to be sure that the goods will keep well, is where good judgment is required. If the packers would supply themselves with a good microscope, with one-twelfth oil immersion objective and an incubator, it would be a very easy matter to know positively if a given process was sufficient. A can or a number of cans from each day's work could be placed in the incubator, and if the sterilization was not complete the bacteria would develop at a blood temperature within twenty-four to forty-eight hours; juice from these cans could be made into a hanging drop, and if any bacteria happen to be present, the microscope would reveal them. Most of the spore-bearing bacteria are motile, and there would be some present, even in small quantities of the juice, long before the formation of sufficient gas to swell the cans. A number of tests could be made with juice of these cans, and the packer could keep close watch on the sterilizing process, and in many cases could reduce the time, which would insure a better flavor.

The exhaust process, or the filling of cans with hot material, prior to the final process, is a good plan for two reasons: The hot

material is expanded by the heat, which produces a vacuum after the cans are sterilized; the heat thus given the material will shorten the final process. The temperature given in the final process varies according to the material and size of the cans. A thermal death point for the bacteria present is first determined, then, to this must be added the time required for that heat to reach the center of the cans; for this purpose the manufacturers of thermometers have made a device, with a self-registering thermometer to indicate the temperature at the center of the can; by repeated experiments the time required for penetration may be known. The mercury tube has a constriction which prevents the return of the metal to the bulb, and when it is removed will show the exact maximum heat at the center of the can. These little devices are inexpensive and should be used by every packer.

We have referred to the vacuum produced by the exhaust process, or filling of cans with hot material prior to the final or sterilizing process; the vacuum is convenient for drawing the tin back to normal shape after it has been swelled at both ends in the sterilization. There was a strong belief among packers that a vacuum was absolutely necessary for preserving canned goods, and there still exists a belief of this kind, not only among some packers, but machinery men as well. The vacuum has no value as a means of preventing decomposition by bacteria. Even if a very powerful vacuum could be produced, it would not prevent the hardy spore-bearing bacteria from developing in cans under-processed. Even if the oxygen remaining in the partial vacuum could be replaced by hydrogen or carbonic acid gas, these bacteria would still be able to grow and multiply. Nearly all of the species identified with spoilage of canned goods grow well in the presence or absence of oxygen, and though aerobes, generally are also facultative anaerobes.

The temperature and time required for sterilization depends upon the nature of the bacteria present, and the character of the material. Different fruits and vegetables vary in their chemical composition. There are also marked differences in the same vegetables and fruits grown in different parts of the world. Tomatoes grown in the Northern States, Michigan, Wisconsin and Minnesota, have less acid and more sugar than those grown in Iowa, Indiana, New Jersey, and Delaware. The different varieties of fruits and vegetables vary in their composition, so that the conditions are different in one locality from those in another. Even in the same locality two kinds of peas or corn, etc., may require different sterilizing processes, owing to the presence on one of a particularly hardy spore bearing baccillus, which may not be growing on the other variety.

It is not possible, therefore, to make a bacteriological investigation of spoilage in one locality and make the conclusions fit the conditions in all cases. One or two bacteriologists have made careful study of sour corn, and isolated a number of bacteria found in the cans, but these bacteria are not always in sour corn; some of them may be found, and even other species entirely different in another location. The same thing applies to peas, beans, asparagus, tomatoes and all kinds of fruits, but these fruits do not vary so much in all their germ flora as do vegetables.

The difference of time and temperature required for sterilizing different kinds of vegetables has been a perplexing problem for canners. It is not generally understood why peas should keep at a temperature below that of corn fully twenty minutes less in time, or why peaches should keep when processed several minutes less than tomatoes.

The difference is due to two facts—there are different species of micro-organisms and there are chemical differences in composition which render the juice of one antiseptic to germs found in the juice of another. As a rule bacteria do not grow in juices which have a marked acid reaction, although there are notable exceptions to this, but generally speaking the more acid the juice contains, the fewer the species of bacteria. The hardy spore bearing bacteria, as a rule, do not thrive well on acid media, even small quantities being detrimental to their growth. The slight addition of sugar however, overcomes the antiseptic properties of some juices, and the spore-bearing bacilli are able to grow to some extent. The writer has often been surprised on opening cans of under processed goods, which were bulged at both ends from the enormous pressure of gases within, to find how few bacteria the juices actually contained. The spores had developed and some reproduction had resulted, until the amount of acid formed had acted as an antiseptic, and multiplication had apparently ceased.

It often happens that the can springs a leak from the pressure, and the gas is liberated, then the bacteria from the air gain entrance and the acids are attacked and reduced to fatty or volatile acids, the original agents having gone into a resting state, or formed spores.

The climatic conditions of certain localities have something to do with the varieties of bacteria found associated with spoilage. In some of the Central and Southern States there are much hardier varieties prevalent than in Northern States, or the New England States, consequently a process that is giving satisfaction in Maine may not be sufficient for sterilizing the same product in Ohio.

Cleanliness, and *proper disposition of waste material,* are prominent factors in sterilization. There are times during the canning season when the raw products, or material only partially cooked,

are exposed to the ravages of bacteria floating on dust, and matter held in suspension by the atmosphere. This is the case when breakdowns occur in the machinery, or when the work is not carried on systematically, or when the receipts of raw material are greater than the canning capacity. The danger is greatest where partially cooked material is exposed to the air. The spore bearing bacteria and the putritive anaerobes are liable to set up *unperceived decomposition,* and elaborate foul substances such as indol and skatol, or may produce bitterness or disagreeable acidity. The partially cooked material offers a more suitable nutrition for these true scavengers, because the cellulose or fibre is softened and the juices are richer in albuminous compounds, which furnish them with all their vital requirements. The danger from these bacteria is not so great where absolute cleanliness is exercised and proper disposition is made of waste material. If the waste material such as cobs, hulls, husks, vines, stems, peelings, seeds and trimmings, are dumped in heaps in the vicinity, the air will be found teeming with the spores of these micro-organisms, ever ready to fall into nutrient material, and there begin their work assigned by nature, the tearing down process of fermentation or putrefaction. If accumulations are allowed to stand from one year to another, the spores of these bacteria may become so dry and hard that the old and tried sterilizing process will fail to be effective. There is great truth in these statements, viz., that age will toughen the spore membrane and that the spore protoplasm will dry and shrink and be more impervious to the action of heat, and require longer time to absorb moisture so necessary for rapid sterilization. Thus the ordinary sterilizing process becomes ineffective. The same danger may lurk in the dirt and dust of the factory; if the floors and machines are not kept clean, by the liberal use of soap, hot water and steam, the harmful bacteria will be present in all parts of the building, in such numbers as to produce chemical changes where least suspected. The evil of uncleanliness is not confined to the breeding of bacteria alone, but flies, insects, rats, mice, etc., are drawn by the opportunities of getting food, and the whole factory will soon be in a very bad condition.

How is it possible to produce fine goods where the entire establishment gives the impression of careless, neglectful methods! Unclean methods breed carelessness in employees, and this becomes evident in the character and quality of the goods turned out. The sterilization is accomplished only at a time and temperature beyond that actually required in a clean, well-regulated factory, and the product has lost the color and flavor which we might suppose once existed.

When Isaac Winslow began to pack corn in tin cans, he used only an open bath process at first. He boiled the cans for several

hours and succeeded, strange to say, in keeping a large per cent. of his goods. Our readers are familiar with the history of his after failures, and his final adoption of the steam retort. That he was ever able to sterilize corn by simply boiling the cans has been a source of wonder to the writer; certainly none of the well-known heat resisting bacteria were present, or if so they had not become accustomed to corn. This theory seems to have some foundation, because bacteria are known to accommodate themselves to certain material when forced to grow in it; for instance, the Bacillus Diphtheria grows very scantily upon ordinary agar, when planted fresh from the false membrane of a diphtheria patient, yet after transplanting upon the same medium a number of times, it grows quite well and if kept in a cool place, loses its virulence to a certain extent. The Typhoid Bacillus becomes less virulent after repeated transplanting, and the same characteristic has been demonstrated in various species. So it may be when corn was first grown in this country that the hardy spore bearing bacteria did not at first find suitable nourishment, except in some cases. Now it is fair to presume that when once started the bacilli became accustomed to corn, and their nature having changed through that nourishment their spores more easily attacked the corn in following years. The open bath process *suddenly* failed and Winslow and his contemporaries lost almost their entire pack.

It is well remembered that corn was sterilized a few years ago by a certain process which is not now effective in all cases, so we cannot but hold to the theory that the most hardy species of bacteria are gradually becoming accustomed to corn, and perhaps other vegetables. No one can say that new species are being created, but such may be the case; if so, then we may be able to explain the necessitated increase of temperature for sterilization. There are, however (found on vegetables), so many species closely resembling each other, differing only in one or two characteristics, that we are strengthened in our first theory, and we may ascribe those differences to the changes in the materials upon which they have habituated themselves.

By increased temperature in the sterilizing process, certainly the color and flavor suffered to some extent, the color particularly, and the demand made by the trade for nearly natural colors in canned goods, forced evil practices upon the packers, corn was bleached and peas were colored with copper, and tomatoes received the "sunshine" from aniline dyes. In some cases the sterilization was reduced, and antiseptics added. This helped to preserve the color, but the quality suffered even more than by increased temperature. In the course of time these methods passed the limit, and some of the goods put upon the market were unsightly, and the

flavor was completely lost. Tomatoes and catsup were colored beautiful carmines, and looked more like red paints than articles of food.

By the excessive use of chemicals and colors to shorten the sterilization, the public turned away from canned goods of this nature, and it is well remembered how the prices dropped to almost nothing (several years ago). The people were getting liberal doses of all kinds of chemicals and colors, in nearly every manu-

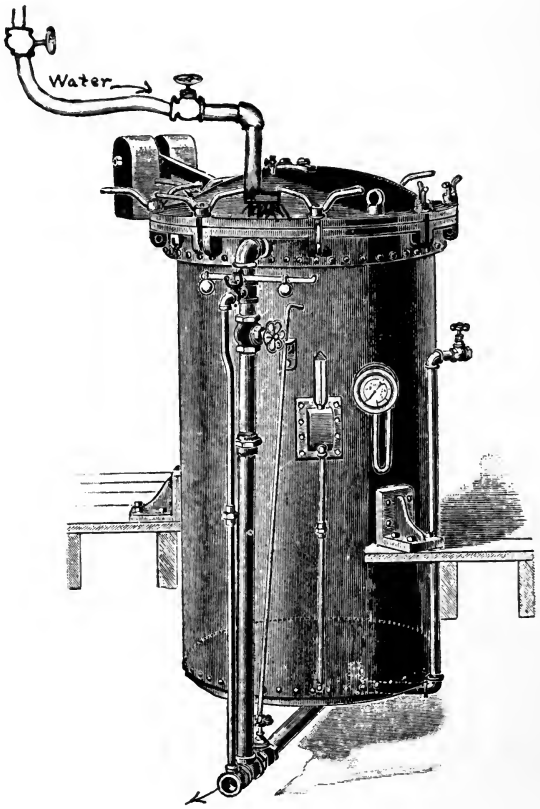


Fig. 31

factured food product brought to the table, so they clamored for pure food laws, elected State Food Commissioners, aided by skilled chemists, and the tide has turned the other way. There is no doubt that these abuses will have settled the lawful right to use certain colors and preservatives, there may be some condiments excepted, but certainly none will be permitted in canned goods, nor are they necessary.

The apparatus for sterilizing canned goods is simple, consisting of retorts with lids which may be sealed; into these live steam is conducted and used either dry or with water and the retorts are exhausted to keep up the circulation, while the temperature is maintained by a thermometer and steam gauge.

For all vegetables, meats, soups and foods of albuminous nature, which furnish all the elements of nutrition for spore bearing bacteria, a temperature of 250° F. is better than any lower degree, for the reason that spores perish at that degree of heat quickly; the time required is variable owing to the numerous complications we have described, viz., the various species of bacteria to be destroyed, the character of the material, and its heat penetrability. Our experience leads us to believe that dry live steam is more reliable than water, because circulation is more thorough, but there is always some danger of discoloration, which may be avoided as follows. A water connection is made with the water line by means of steam hose, to the lid of the retort, and a large overflow is made near the top of retort, so that a considerable volume of water may be let into it, just as the temperature drops to about 220° F. after the cans have received a full process. The sudden rush of cold water chills the goods, and stops the cooking which is still going on within the cans. Otherwise when the cans are thus cooking, and the lid is opened suddenly, the air striking the cans will cause discoloration, but if the retort is completely filled with cold water (before opening the lid), the color will remain good, and there will be no scorched taste unless the sterilization has been unnecessarily prolonged. 250° F. is equal to about fifteen pounds pressure and may weaken the seams of the cans unless the chilling is done (with water) before their exposure to the atmospheric influences.

Another method of sterilization coming into favor is the calcium process, which in some measure seems like a return to ancient methods, yet improved as it is, has some advantage over the ordinary steam retort system. The principle of obtaining a higher degree of heat than boiling water in a fluid, is based on the specific gravity of the fluid. By adding calcium to water the specific gravity is increased until temperatures of 240° and even 250° F. may be obtained, without ebullition, consequently there is little or no escaping steam, and the temperature may be maintained at a very small expense, when compared to the cost of generating steam for retorts.

The latest device consists of a long tank filled to a certain depth with calcium water, through which the crates containing the cans are dragged on a carrier, which is slowly speeded to give the required time for sterilization. After the cans are sterilized, they are carried through running water to cleanse them, and this also stops

the cooking which is going on inside the cans after they have passed through the calcium bath. This process has the advantages mentioned above, being a fuel and labor-saving method, but it cannot be used successfully for sterilizing glass goods, which require high temperatures. Glass goods are difficult to sterilize, owing to the breakage and the small expansive properties of glass. Glass goods of all kinds may be sterilized with dry steam at any temperature up to 250° F., if the precautions are taken to raise and lower the temperature very slowly. The successful sterilization of glass goods depends largely upon the quality and thickness of the glass, also the method of sealing. *The French have been importing all kinds of fruits and vegetables* put up in glass, but nearly all such goods are preserved with antiseptics of one kind or another, and while they look well, *are not to be compared to the goods packed by many American canners.* Glass seems to have the advantage over tin, in the opinion of many persons, because there is a mistaken and widespread belief that there is some danger of metallic poisoning from tin and the solder and flux used in sealing. It is a question, however, if there be more metal in tin goods than in some of the highly-colored French glass goods sold on the market.

The writer has made a number of analyses of various canned goods for tin, lead and zinc, and the quantity of the first two are almost incalculably small. The most definite amount of tin and lead were obtained from canned pineapple and California fruits, but no appreciable amount was obtained from these. Some samples showed the presence of more or less zinc, due to careless use of soldering solution. The old method of brushing the caps with an ordinary paste brush dipped in zinc chlorid has given way to the beautiful little machines which carry the solution on pencil brushes around the crease of each can, allowing none to be sucked in through the vent hole.

Of course, glass goods, if packed with care and without the use of preserving agents and metallic colors, are entirely free from metals, excepting where they are found in composition. Some vegetables have iron and copper in composition. Tomatoes grown in Western Pennsylvania contain some copper.

The sterilization of goods in glass imparts a slightly scorched taste, because it is impossible to chill with water. The cooking goes on until the temperature drops below the boiling point.

Another method of sterilizing canned goods has found favor among the packers of canned meats and special food products. The apparatus is a monstrous affair requiring a great deal of room. The cans are placed in the carriers and allowed to pass through oil, which is heated to a certain temperature. This apparatus is used by the Armour Packing Co. of Chicago, and the results are

very satisfactory. After the cans pass through the oil, they are drained and then carried through an alkaline solution, and then through running water.

There are several methods employed now to avoid the old "venting" process and even in the canning of meats this is entirely obviated. The object of the venting process was two-fold, viz., to heat the contents of the can to obtain a vacuum, and to drive off unpleasant gases. Outside of tomatoes, there are hardly any products cold packed unless special apparatus is employed. For ordinary canning all that is necessary is to heat the syrup or liquid which is used to cover the fruits and vegetables, or to heat the vegetables before filling into the cans; corn is thus heated before the filling.

There are several devices used to pack certain goods without previous heating or "venting"; all these are modifications of the vacuum process. The cans are passed into a machine which exhausts the air by a vacuum pump, and while in vacuo are sealed. The machine used in packing meats is interesting, because the "tipping" or "dotting" is done in vacuo. The machine is round and carries the cans in 15 pounds vacuum, under a glass window; the inside is illuminated by incandescent light, and a small round disc of solder is melted by a copper, which is heated by electricity. The operator can look down through the window and melt the solder over each hole as the cans pass; when all have been "tipped," the vacuum is released and the cans pass out, when they are inspected for leaks.

The old method of venting canned meats was difficult, costly and uncleanly. The cans were filled cold, and a piece of tin was bent and soldered onto the under side of each cap to prevent the meat from coming up into the hole, which was made with an awl after the cans were heated the first time. The juice and grease would squirt all over everything when the cans were punctured and the closing of the holes was quite difficult, requiring skilled help.

There are several kinds of vacuum machines on the market, but they require special cans. The lids of these cans are prepared with patent cement. The cans are put into the vacuum machine with the lids loosely placed, and when the air is exhausted the lids are forced down into place and the cement holds the vacuum in the cans. The cans are made without soldering, except the body seam, the sealing being completed by crimping the flanges of the body together with the rims of both tops and bottoms. The cement coming between the two edges prevents leakage.

These machines are used in European canneries and give good satisfaction. Recently, improved machines have been built in America for making these cans. These cans are also coated with a

substance which is not attacked by fruit acids. The inside coated cans are preferable for goods of delicate color. The ordinary cans are thus coated also.

There is another method of sterilization used largely in bacteriological work to destroy the bacteria in culture media, which become altered by high temperatures. It is called "Discontinuous Sterilization" and was discovered by Prof. Tyndall during his experiments and efforts to overthrow the theory of "spontaneous generation," advanced by Von Liebig and his contemporaries.

Prof. Tyndall experimented with all kinds of infusions made from meats, vegetables and grains, trying various degrees of heat from 212° to 300° F., and prolonging the time for hours. These experiments are recorded in Tyndall's "Floating Matter of the Air." There is no question but that his experiments at 300° F. were faulty wherever he failed to sterilize his infusions in an oil bath. We have conducted numerous experiments, using various temperatures, and up to this time have never discovered any species of bacteria whose spores could withstand 250° F. moist heat for more than a few minutes. This statement must not be applied to the sterilization of canned goods, nor any material which is impervious to heat; it applies only to that temperature directly upon the spores, which are in solutions or materials easily penetrated by heat.

Tyndall's infusions were sterilized in glass flasks having a thin tube for escaping steam, which was sealed by melting the glass together while the steam was escaping. This would not be reliable, since the temperature must be lowered to melt the glass together, or else there would be a fine hole in the glass, which would be an entrance for bacteria from the air. If the infusions were cooled to allow the perfect sealing of the glass, a vacuum would form in the interval and spores from the air could find entrance.

There is no question but that 250° F. moist heat for twenty minutes will destroy all known spores, if the necessary precautions are taken to avoid contamination from the air. It is marvelous how a small hole in the soldering of a can will be the means of spoiling the contents. Holes which are as small as a needle point, are wide open doors for such infinitely small vital powers as spores, and it is often difficult to find some of these leaks in spoiled cans. Through a hole the size of a period (.) 50,000 spores could pass side by side into a can without touching the metal.

Discontinuous sterilization is conducted as follows: Moist heat, usually 212° F., is applied to the material in water bath, for a time ranging from twenty minutes to one hour, after which it is placed in a cool place for one day, and the same process is then applied a second time, and this is continued for three days, so that the material receives four processes, which render it sterile.

The scientific principle is based on the time required for spores to vegetate. As we have stated in previous pages, the vegetating cells are easily destroyed; very few, indeed, are able to withstand 180° F., and almost all perish at 165° F. Now the spores, being very resistant to heat, are simply softened during the first one or two processes, and readily perish when they begin to vegetate. Probably most of them are destroyed in two heating, but a few of the drier forms may require a day or two longer to swell up and vegetate, so that the four heatings will as a rule destroy all.

As we have stated, there are certain materials which are chemically altered by high temperatures, and it is necessary to resort to the discontinuous method in order to prevent undesirable changes. Gelatin loses its solidifying properties if heated to 230° F. Milk is very much altered when heated even to boiling temperature, there being formed such substances as formaldehyde, dioxygen and calcium citrate, the latter being thrown down as a precipitate. Milk is therefore sterilized at about 165° F. for one hour, on five consecutive days, to avoid these alterations.

There are constantly made improvements on all methods of work and we believe that the discontinuous sterilization of canned goods by practical means offers a good field for experiment and study. On a small scale in laboratory work it is successful. All kinds of infusions of meats and vegetables are easily sterilized (in test tubes and flasks) by simply plugging the necks with cotton and heating in the manner just described. The color and flavor of such vegetables as corn, peas, string beans, asparagus, cauliflower, lima beans, etc. (when sterilized discontinuously), are very much superior to those of the regular canned goods, which have received 240° or 250° F. The writer has made a number of such experiments and the results are gratifying.

Sterilization by electricity, such as a direct current or an alternating current or the X-rays is not reliable, and by the first two certain chemical changes are produced which are undesirable. It was hoped and claimed that the X-rays would accomplish sterilization by causing paralysis of the bacterial cells, but repeated experiments have demonstrated only failures.

Sterilization by heat is the only method of value for the canning industry. There are some special products which are preserved by chemicals, but these will no doubt be regulated by pure food laws in such a manner that only a minimum quantity of preservatives will be permitted, or perhaps none.

One fact must not be lost sight of, however, and that is that various fruits and vegetables contain within themselves certain acids and salts which make them easy to sterilize by heat; these elements of composition act as antiseptics and in some cases as disinfectants for many of the spore-bearing species of bacteria.

It must not, therefore, be inferred that a certain process by heat actually destroys all life, but that all bacteria are prevented from multiplying through the destruction of some (by heat) and the antiseptic power of the juices. This may hold good so long as perfect fruits and vegetables are used and the complete elimination of all foreign matter is observed, but should unsound material be used or adulterations find favor, or should dirt or foreign matter get mixed in with good material through carelessness, the conditions will be changed, and the regular heating may not prevent decomposition. By avoiding adulterations and observing the strictest rules of cleanliness losses will be minimized.

Certain fruits contain antiseptics, as we have stated, and certain antiseptic compounds are formed by oxidation of sugars and fats, by the influence of light and at 250 degrees F. These antiseptics play a very important part, as we have shown in sterilization, and it is our purpose to study the various compounds found in canned goods, and also the various antiseptics which are used to accomplish sterilization in special food products.

CHAPTER VII.

Preservatives

What are Preservatives?—Preservatives are not Ordinarily Used in Canned Goods.—Some Food Products Require Them.—Natural Origin of Preservatives in Food Products.—Statements made by Various Authorities Analyzed and Criticised.—Sterilized Catsups, Preserves and Fruit Butters not Satisfactory to the Trade.—Some Opposing Arguments Answered.

It is well known that certain chemicals when added to food have a restraining influence upon the bacteria, yeasts and molds which are associated with its decomposition. Some chemicals prevent the multiplication of bacteria and are called antiseptics, while others will kill the germs and also the spores and are classed as disinfectants. It is difficult, however, to draw the line between antiseptics and disinfectants, for the reason that large quantities of the chemicals known as antiseptics, may also prove to be germicidal and therefore disinfectants, and, on the other hand, small quantities of chemicals known to be germicidal, may not destroy the life of bacteria and therefore only antiseptic. Some antiseptics when used in limited quantities favor the growth of certain bacteria and are used in obtaining pure cultures; the chemical will prevent the growth of some varieties which ordinarily overrun the culture plates. The term antiseptic is applied to a number of chemicals which are used to some extent in the preparation of foodstuffs, principally catsups, sauces, preserves, etc. They have been found in canned goods in some cases, no doubt, purposely added by the packers to shorten their sterilizing process, but more commonly due to natural causes.

The harmful nature of the various chemicals has been argued pro and con by the best exponents of science, but their effect upon the human body is disputed, and the actual effect of some is not known. They may be harmful or they may not be, but they are considered (by the authorities) as unnecessary, and "the burden of proof, therefore, rests upon those who employ them." If they are not harmful, then they may be classed as adulterants (if used in canned goods) from the fact that they are largely unnecessary and do not add any nutritive value to the food. If they are harmful, there is sufficient ground for prohibiting their use. There can be no possible argument for the employment of chemicals as antiseptics in canned goods.

I do not find that they are used to any great extent in canned goods, the methods of former years having been superseded by the more reliable and less expensive sterilization by heat only. There may be some packers who use chemicals for preserving canned goods, but they are very ignorant of their business. The best houses sterilize all canned goods by steam heat either directly or indirectly, and do not increase the cost by adding expensive antiseptics.

Some of the published reports of antiseptics found in various brands of canned goods are absurd and misleading. There have appeared reports indicating that as many as three different chemicals were found in a single can. On the face of it, it is most unlikely, because no packer would be so foolish as to waste his money in this manner, and even if he should use the chemical method he would hardly use more than one as an antiseptic.

Antiseptics have been too freely used in some kinds of food in the past and adulterants have been added to so many varieties of food that the public has rebelled and food commissioners have been employed to fix the responsibility upon guilty manufacturers and laws have been passed to prevent such products from being exposed for sale. Some of the reports made by analytical chemists will show to what extent this has been practiced, and while they may be overdrawn in cases, yet there is no doubt that such practices have gone beyond the limit of tolerance.

It was claimed by one very radical food commissioner that it was no uncommon occurrence for a person to sit down to a meal having the following list of chemicals included in his diet: "Ham containing saltpeter; canned corn containing saccharin, salicylic acid and hyposulphite of soda; canned peas containing copper and alum; tomato catsup containing benzoate of sodium and coal tar dye; wheat cakes containing ammonia or alum; maple syrup made from cane sugar syrup and glucose; mustard containing tumeric; milk containing formaldehyde; coffee artificially prepared from various substances; pepper containing ground cocoanut shell, and butter containing borax. Verily one would have an internal drug house if this were continued day after day." It is hardly likely that a person would be so unfortunate as to meet with all these compounds in a single meal, yet it might happen that he would be confronted by a few of them at least. The claim is made that such combinations must in time cause trouble in the human stomach, because the juices which enter into the digestive processes were never intended to perform the work of an analytical chemist three times each day. If such conditions actually existed there would be some ground for complaint, but canned goods packers are not responsible for these adulterations. There are some points worthy of consideration

when dealing with the question as to whether antiseptics shall be prohibited in all food products. It is well known that bacteria are scavengers and more readily attack food which has been cooked, than food in the raw state; the cooking seems to soften those foods containing fibre and releases the nutrient juices and fluids upon which the bacteria find suitable elements for growth. Bacteria which produce ptomaines and toxins and disease parasites also flourish upon cooked food, and if that food is not consumed as soon as it is exposed, it may prove more dangerous than if it contained an antiseptic. There are many food products which are not eaten alone, but are used to give flavor and relish to other articles of prepared food. Such products may not be entirely consumed at a single meal and may be regarded as luxuries. Now, if any of this class is subject to decomposition it would seem wise to use a certain per cent of antiseptic to preserve it. Under this head might be mentioned such articles of food as butter, cheese, tomato catsup, Chili-sauce, apple-butter, peach-butter, and other sauces and relishes. The argument has been advanced that such foods may be prepared in small size packages, and thus avoid the necessity of carrying over from one meal to another, but the expense would be heavy, and in many cases would not be practical, especially for hotels and restaurants. This is a question worthy of careful consideration and we believe that the manufacturer has a shade the better of the argument.

The great mass of our people do not care to waste money on glass or tin packages of small size. They want as much as possible of the contents, and as little as possible of the package in their investments, and we believe that if a strict ruling were made against the employment of preservatives, it might prove to be a burden on the people or else would bar the masses from using these luxuries. It is difficult to draw the line, however, and a thorough test should be made of all modern preservatives to determine positively their effect upon human beings in the quantities ordinarily employed in food products. The honest manufacturer is ready to comply with any good national pure food law, so long as he is protected by that law.

There are some facts concerning antiseptics which are quite necessary to be known, and it was the author's privilege to bring them out at the convention of state food commissioners and chemists at St. Paul, Minn., in July, 1903. Various antiseptics are formed naturally in fruits and vegetable. Various antiseptics are formed during the processes of manufacture, especially where fermentation is employed, and where sterilization is accomplished at high temperatures. Antiseptics are formed where certain food products are exposed to strong sunlight.

Prior to this convention there appeared published reports of analyses made by different state chemists who claimed to have found various antiseptics in canned goods. Knowing that certain brands of goods had been packed absolutely without the employment of these antiseptics, we began a series of analyses to determine their origin. We found that the chemists' reports were true in only a few cases, but we also found that antiseptics were formed in the manner stated above. The chemists who had been conducting the analyses for the people were pleased to learn the results of our investigations, which placed the canner in a far better light than ever before. We believe that much good was accomplished at the convention and that the chemists and manufacturers understand each other better; both sides were fair and willingly heard the arguments pro and con.

There are various fruits and vegetables which contain certain antiseptics. Whortleberries contain 0.6 to 0.8 gram of benzoic acid per liter (Lafar's Technical Mycology, Section 80).; raspberries contain salicylic acid or phenol, as also does horseradish, which will prevent the acetification of cider in one part to three hundred and fifty. Currants contain benzoic acid and salicylic acid. Cherries, plums, crabapples, grapes, strawberries, apricots and peaches, contain salicylic acid in appreciable quantities. An analysis of cranberries was made by the author to determine the quantity of benzoic acid present naturally in them. From 125 grams of cranberries we obtained 60 milligrams of the preservative. This is equivalent to one part to 2080, and we are quite sure that there was still more which could not be extracted. This is a very large amount of preservative to be found naturally in one of our finest fruits. In Ohio or Pennsylvania any one who sells cranberries is liable to be arrested and fined for selling an article of food containing a substance which is poisonous or injurious to health, according to the rulings of the pure food commissioners, and the decisions of the courts. Some of the jams, preserves, fruit butters and jellies do not have any more of this preservative than occurs naturally in cranberries. Why are cranberries so valuable and so much relished with turkey dinners? Is it not because the benzoic acid assists digestion, and also prevents any decomposition of the food which the stomach cannot quickly take care of? The benzoic acid prevents the bacteria from multiplying until the stomach catches up with the unusual amount of work forced upon it.

Analyses were made of two lots of green gages to determine the presence of benzoic acid. These two lots were obtained from different places, which makes us reasonably sure that no benzoic acid had been added artificially. 500 grams were used, or about one pound for each extraction, and the seeds were broken. After preparing the fruit for the chloroform, several c. c. of 25 per cent sul-

phuric acid was added, although this is not necessary. The fruit was then placed in a separatory funnel and 125 c. c. of chloroform was added and it was shaken up well several times. After a time the chloroform was drawn off before the formation of an emulsion. The extract was divided into three parts and after spontaneous evaporation tested for benzoic acid with the Ferric chlorid test, and benzoic acid was found. Mohler's test was then used, converting the benzoic acid into metadiamidobenzoic acid, which confirmed the first test. Both samples of green gages gave the reaction. Salicylic acid has been found in many different fruits as shown by the

REPORT FROM MONTANA EXPERIMENT STATION.

"Among the fruits from which we have obtained the salicylic acid reaction are the following: Strawberries, raspberries (both red and black), blackberries, currants, plums, black cherries, apricots, peaches, Concord grapes, crabapples, standard apples and oranges. In a few instances we have this work quantitative with the following results:

Currants, 0.57 mg. acid per kilo of fruit.
Cherries, 0.40 mg. acid per kilo of fruit.
Plums, 0.28 mg. acid per kilo of fruit.
Crabapples, 0.24 mg. acid per kilo of fruit.
Grapes, 0.32 mg. acid per kilo of fruit.

These values, however, are not absolute, but only comparative, and represent the amount which we succeeded in extracting in each case. We distilled the fruit with phosphoric acid, extracted the distillate with ether, took up with small amount of water, and applied the ferric chloride test after the ether had evaporated. Check analyses made with known amounts of salicylic acid showed that nearly all of the acid was extracted by this method. We have also found the salicylic acid reaction to be given by tomatoes, cauliflower and string beans.

It seems to us that the bearing of this work is very important, particularly as regards the investigations of food chemists. While these very small quantities may not react to the test for salicylic acid as usually applied, especially in view of the small amount of material generally worked upon (25 grams), yet a knowledge of its wide distribution may save reporting, on occasions, materials as adulterated to which salicylic acid has not been added. Knowing that salicylic acid may occur in many of the substances, either a quantitative determination will be necessary in each case, or it will be well to report only on strong reactions.

We were led to this investigation by the protest of a well known reputable firm, in whose currant jelly we reported salicylic

acid, but which was present in no greater quantity than we have since found in fresh currants. A similar experience was lately had in one of the state laboratories for food control.

In addition to the above work we are studying the distribution of benzoic acid in fruits and vegetables, and hope to be able to publish our results within the year."

Signed,

F. W. TRAPHAGEN,
EDMUND BURKE."

Journal American Chemical Society.
March, 1903.

Tomatoes and acid fruits contain antiseptic properties in their juices. Formaldehyde is present in minute quantities in almost all foodstuff, which is exposed to the action of micro-organisms even for a short time, and the official sulphuric acid test will show it often I have seen milk drawn from the cow's udder and tested for formaldehyde, which gave a positive chemical reaction. There are a number of raw materials which undergo partial fermentation before they are worked into finished food products. During that fermentation there are formed various chemicals which are elaborated by bacteria; such as phenol, formic acid, sulphites and nitrates, and enough of these substances may be present in the finished goods to give the reaction in the official tests. Among the products which undergo fermentation as a part of the manufacturer's process are pickles, olives, onions, sauerkraut, tomatoes for catsup, cauliflower, herbs, garlic, soaked goods such as navy beans; pickled meats and various other raw materials.

There are formed in some canned goods during sterilization at 250° F., such compounds as formic acid, formaldehyde and dioxygen, which are due to the oxidation of fats and sugars. Some of these are formed always in canned corn, principally formaldehyde. In the presence of sunlight also certain raw materials and even finished goods will form chemicals having strong antiseptic properties, dioxygen being the most common (Novy's Laboratory Bacteriology, page 70). Milk, when heated only to 212° F., will show the presence of formic acid and formaldehyde, and at 250° F. these give a very marked reaction, and the milk undergoes chemcial changes, such as the precipitation of calcium citrate and the formation of dark fission compounds having an empyreumatic flavor. (Attested by numerous authorities.)

Now we must not understand by all these statements that enough of these antiseptic compounds are formed to arrest decomposition; indeed, such is the case only with a very few, and even in those, decomposition will eventually take place, but these facts are

brought out to show that there may be some ground for the chemists' report on certain goods when they state that these antiseptics are present. On the other hand, these facts will serve the chemists and should enable them to make allowances for products formed during the process of manufacture, or those which have a natural origin in raw material, used in making up the finished food product. By conducting control tests and making a careful study of raw material, the chemist will be in a position to state positively if antiseptics are purposely added, or whether they are of natural origin.

From the very fact that nature produces so many examples of the natural formation of compounds with marked antiseptic properties, it would seem that the manufacturer might be allowed to follow nature's example in some cases.

So much has been written and said on the harmfulness of preservatives in food within the last ten years that any further discussion might seem unwarranted. However, the subject is one of such vital importance to manufacturers of certain Food Products and Table Condiments, that any information of importance cannot fail to be interesting. A few years ago the warfare waged against the employment of salicylic acid became so strong, that laws were rapidly passed to stop its use in every article intended for food and drink. After the passage of these laws, the writer was one who strongly urged the manufacturers to cease using the chemical. At that time the question was one of obedience to State authority, however dictatorial it might seem. In the absence of means and data to refute the charges made by famous medical and scientific authorities, the manufacturers saw the measures presented and passed against them without being able to oppose them. I have always stood for strict obedience to the laws governing the employment of preservatives in Canned Goods, and so far as possible in other food products, but we need a thorough test of the statements made by the authorities who were responsible for the measures set against preservatives.

Personally, *I believe that salicylic acid one part in one thousand is not only non-injurious when employed in foods and drinks, which are necessarily exposed to the action of bacteria, but is positively beneficial, having a tendency to ward off intestinal diseases, such as typhoid fever and cholera.*

I do not wish to be misunderstood in this discussion of preservatives. So long as the laws prohibit the sale of or exposing for sale of goods preserved by means of salicylic acid, let us as manufacturers, by all means live in obedience to them, and if we desire a change in the statutes, let us go about it in a way that will overthrow the statements made by those authorities who are responsible for the enactment of such laws. Our first step therefore will be to

analyze the statement of these authorities and present facts which shall overthrow any theories, and if not entirely successful, to at least throw some shadows of doubt on them. It is an old saying that a statement never loses anything by repetition; on the contrary, it gathers force and volume as it is repeated, so that a simple doubtful statement is sometimes expanded by frequent misquotation into an apparently positive fact. In the world of science we find that much of the literature is merely a copy of a true investigator's work, and we are mystified frequently by statements directly opposite coming from supposed authorities. Much of the scientific literature of the time should probably never have seen the light of day, because the statements made are sometimes not borne out by facts. We find this state of affairs existing in the field of science, where a writer presumes to quote some other writer as an authority. Probably the preceding writer misquoted an investigator, and a positive error is put down in a text-book under the authorship of one, whose titles protect him from vulgar criticism, when the fact is he was perhaps too preoccupied to make the investigation for himself.

Dr. J. Dixon Mann is one of the most bitter opponents of salicylic acid in food and drink, and his evidence was accepted by the *British Parliamentary Inquiry Commission*, and is a sample of much similar evidence also accepted. I make the quotation in full so that no mistake may be made in the discussion: "Last year in the summer, at lunch in the club, I took to drinking cider and continued taking it for many weeks. I began to feel a peculiar tendency to looseness in the bowels; furthermore, I felt never, as it were, thoroughly relieved after motions. This went on for a time and I could not understand how it was. I thought it was accidental in the first place, but it kept going on week after week. I did not care to take any medicine and I began to cast about for what possibly could be the cause of it. I went over the things I had been in the habit of taking, and the things I was taking at the time. I could not think of anything until it struck me about this cider; so I got a bottle of the same sort from the steward of the club, took it to my laboratory, and found salicylic acid in it, and, needless to say, I have not taken cider since." I want to call particular attention to the last few clauses of this testimony: "And found salicylic acid," etc. Why did he not look for malic acid, succinic acid or some other substance? No, he found salicylic acid and jumped to the conclusion that it was the cause of his particular complaint. If he had shown a true scientific spirit, is it not reasonable to suppose that he would have made a quantative analysis of the salicylic acid contained in the usual amount of cider which he was in the habit of drinking each day? After a time, when he was in perfect health, he could have taken the same amount of the acid mixed with other food, and then obtained more direct evidence.

It is a source of wonder to me that such an eminent body of men as composed the Parliamentary Inquiry Commission should accept such evidence.

The demand for preservatives to prevent fermentative and putrefactive processes in certain kinds of perishable food is so great, and the interests involved are so enormous, that snap judgment should not be taken against them.

I have always written against the unnecessary employment of preservatives in canned goods or other foods which may be sterilized by heat only, for the reason that it is expensive, unnecessary, and it is not wise to overdo the thing. It would not be wise to preserve everything with salt, it would not agree with us to have too much of it—harmless and necessary as it is, but we are quite satisfied to have our hams, sausage, pickles, etc., so preserved. It would not be wise for us to preserve all our food with sugar, it would soon make us all sick, but we are satisfied to preserve our fruits, jellies, jams, etc., with it, because at times we relish the change of diet. It would not be wise for us to preserve all our food by smoke (which is an application of creosote, phenol, etc.)., but we are satisfied to have some of our meat put up in this fashion. It would not be wise to preserve all our food by sterilization in hermetically sealed packages, because we want a change, yet we are satisfied to have a great variety so put up for winter use. Therefore an indiscriminate use of salicylic acid would be unwise, but when restricted to such foods as are subject to chemical changes by bacteria, it would be the part of wisdom to permit its use, provided it is not injurious to health.

It has been so declared by a number of authorities on the following grounds:

1. "It is an antiseptic and anti-fermentative, and is therefore liable to interfere with the digestive processes by destroying the digestive ferments."

2. "After absorption it is apt to injure the general health, and to interfere with nutrition."

3. "It is an irritant, and is therefore apt to injure the mucous membrane of the stomach and intestinal canal."

In the report of the Department Committee appointed to inquire into the Use of Preservatives, etc., presented to both Houses by command of His Majesty, London, 1901 (page 96), is the testimony of Dr. Robert Bell, Fellow of the Faculty of Physicians and Surgeons of Glasgow, and Fellow of the Royal College of Surgeons of Edinburg. He stated that he and his family had knowingly and regularly taken food containing antiseptics for eighteen years and without a sign of harm to any of them. "During the whole of that period we have never had a single case of illness in the house; when scarlet fever, measles, whooping-cough and other ailments were

rampant, there was not a single one of our children ill. There is another family that I know very much in the same position as ourselves."

In papers read before the Liverpool Medical Institution on November 20, 1902, by Dr. C. J. MacAlister, of Edinburgh, and Dr. T. R. Bradshaw, of Dublin, they gave their experiments with salicylic acid on digestive processes, and the digestive ferments. Quoting their report fully they said: "In this inquiry we have nothing to do with the organized living ferments (bacteria, yeasts, molds, etc.). ; these are certainly killed by salicylic acid, and its efficacy as a food preservative depends upon that very fact; but we have found that the digestive processes will proceed in the presence of the acid even in a solution of 1 to 500, which is practically saturated.

Our first experiment was made with pepsin which, if active, should dissolve 2,500 times its weight of hard-boiled egg, and the Pharmacopoea provides the following test for its activity: "If 12.5 grams of coagulated and firm white of egg, 125 c. c. of acidulated water containing 0.2 per cent muriatic acid, 0.005 gramme of pepsin be digested together at 105° Fahr. for six hours and shaken frequently, the coagulated white of egg should dissolve, leaving some flakes in solution. Having ascertained the activity of a specimen of pepsin by this experiment, we repeated it in two flasks each containing the above specified ingredients in their proper proportions, but to one of the flasks 0.250 gram of pure salicylic acid (which had previously been dissolved in eight c. c. of boiling water) was added, and at the end of six hours it was found that in the flask containing salicylic acid, there was only a small amount of white of egg left, none being left in the other flask." They go on to say, however, that the addition of salt to a tube containing pepsin and white of egg gave practically the same results, then they sum up the results of their experiments in words as follows: "We have found that salicylic acid exerts about the same retarding influence on the digestive processes, as do many articles, such as kitchen salt, which are always present in a mixed diet. . . . the question therefore is not whether salicylic acid delays digestion at all, but whether it does so to a greater extent than other bodies, such as kitchen salt, which form part of an ordinary diet."

The experiments of these two eminent authorities bring out the fact that salicylic acid does not interfere with natural digestion, and makes a clear distinction between organized or living ferments, such as bacteria, yeasts and molds, and the unorganized ferments or digestive enzymes, pepsin being most prominent. The opponents of salicylic acid have never shown the proper spirit of investigation,

but rather a kind of theoretic deduction, jumping from one hypothesis to a conclusion without the solution somewhat after this style.

HYPOTHESIS—Salicylic acid destroys bacteria which are ferments.

CONCLUSION—Therefore digestive processes are impeded because they depend upon ferments.

We can readily see, therefore, how false the conclusion must be if it is made to depend upon the hypothesis, where the bacteria are called ferments without differentiation from unorganized ferments such as pepsin. We know that the living ferments are destroyed by the change produced in the cell protoplasm, resulting in plasmolysis, but in the unorganized ferment there is no cell protoplasm and the action of the preservative cannot therefore be the same.

It should also be borne in mind that the opponents of salicylic acid have generally taken abnormally strong solutions of salicylic acid, to maintain their claims. No person ever takes this acid at meal time as strong as a cold water solution, yet this is a favorite (amount employed in experiments,) by those who would convince the people that they are dying of slow poisoning.

An official of the Department of Agriculture recently stated that "The burden of proof, that preservatives are harmless to man, rests with the manufacturers who use them." Such a statement, when closely analyzed, is a deduction drawn from the testimony of various medical authorities which have declared that preservatives are poisons and injurious to man. It implies that since preservatives have been so declared by those who ought to know, direct evidence to the contrary must be produced by manufacturers who use them. Therefore, some of them stand condemned as harmful, because many authorities have stated that in their opinion such was the case.

The task of proving the fallacy of many statements advanced by the opposition is no easy one. The public mind is already to some extent prejudiced against preservatives in food, simply because some authorities have made the assertion that they were harmful. There are a number of condiments which are commonly preserved with such chemicals as salicylic acid, benzoate of sodium, and borax, the last two particularly. The condiments so preserved may be sterilized by heat only and they will remain pure and unfermented as long as the container is hermetically sealed. As a rule these condiments have a very delicate flavor which is greatly injured by a sterilizing process sufficiently prolonged to destroy the yeasts, molds and spores of bacteria present in them. I have been conducting a series of experiments with such goods for the past six years. Many carloads of condiments put up in glass and sterilized

by heat only have been sent out to the trade. It is a remarkable fact that such goods have failed to give general satisfaction for three reasons. They have a slightly scorched taste, or a pasteurized taste and odor. The natural color is somewhat darkened and the goods soon spoil after the containers are opened. We have found that tomato catsup which has been sterilized by heat only is greatly injured in flavor and will not keep for more than five days after the bottles are opened. Frequently fermentation sets in about the third or fourth day and mold will be visible to the eye in four or five days.

Another remarkable fact brought out in these experiments was the preference shown by the consumers for condiments prepared with preservatives over the same goods sterilized by heat only. In many cases the facts were made clear to the consumer. The consequence was that much of the pure goods still remains unsold on the grocer's shelves, while those prepared with preservatives are selling rapidly.

Catsup both with and without preservatives, has been placed throughout Kentucky, Minnesota and the Dakotas, and a large per cent of that sterilized by heat only remains unsold, and the trade has been greatly injured owing to the loss of flavor.

Now let us examine the reasons for this difference in flavor. Why is it that the flavor is injured more by heat than by preservatives. It is well known that preservatives destroy flavor, especially if used in excess, and we know that heat will not injure the flavor very much unless it is prolonged.

In preparing table condiments with preservatives, the manufacturer does not plan to destroy the organized ferments such as molds, yeasts and bacteria, but to prevent their multiplication or growth. The chemical changes caused by fermentation are produced during the multiplication of the organized ferments. The elements necessary for the multiplication of ferments are obtained from the carbohydrates and other complex substances, and there are formed new chemical compounds greatly differing in taste and odor from the original substance. Therefore preservatives are added in just sufficient amounts to prevent the multiplication of these ferments. No attempt is made to destroy them, because, as a rule, they are non-pathogenic and are not harmful to the human organism in small numbers. Very few of the preservatives ordinarily used in preserves, apple-butter, tomato catsup, Chili-sauce, etc., are antiseptic, strictly speaking, and certainly they are not germicidal in the quantities used. This accounts for the spoilage of such condiments after a time, because ordinary preservatives finally lose their preventative power.

Sterilization by heat only is a far different problem. All spores of yeasts, molds and bacteria must be destroyed absolutely. One

or two spores are just as dangerous as millions, if they remain alive. From one spore there will spring into existence many millions of the same species within a very short time, where the temperature and other conditions are favorable. In order to completely sterilize table condiments considerable heat is necessary, owing to the density of the goods and the size of the container. Goods of this kind are usually sold in glass, because tin is not suitable for them, owing to their high acidity which attacks the tin plate vigorously. Sterilization requires boiling for perhaps thirty or forty minutes to destroy all spores. The organisms near the outside, of course, perish quickly, but those in the center do not get the required temperature for a considerable time, varying with the diameter of the container and the density or penetrability of the goods. Certain portions of such packages, therefore, receive more heat than is necessary for the destruction of ferments, and of course, the flavor suffers accordingly. If such goods could be heated uniformly, the loss of flavor would still be greater than in the same goods containing preservatives sufficient to inhibit bacterial growth, because complete destruction is necessary in the one, while inhibition only is necessary in the other.

Our claim for the necessity of preservatives in food of slow consumption is a good one, because it has been demonstrated that the people prefer such goods with as near the natural flavor as it is possible to make them, and sterilization by heat does destroy much of the original flavor. Our conclusions are thus summed up:

1. The ordinary preservatives employed for preserving goods of slow consumption are valuable and necessary, because in no other way can the original flavor of such goods be retained.

2. The consumer prefers this class of goods even when he knows that preservatives have been used to keep them in an unfermented state.

This does not dispose of the question, "Are such preservatives harmful to the human organism?" but it is encouraging to note that the consumer is better pleased with table condiments so preserved.

The testimony offered before the Parliamentary Committee by a large number of physicians and professional men is interesting, and is remarkable for its utter lack of evidence based on experimental investigation. One physician after another is called before the committee, and nearly all evidence is founded on mere opinion or the result obtained by abnormal quantities of preservatives. True investigation had not been made by many, and those who had done any experimental work did not produce the notes and data to prove their claims. The following testimony was offered as evidence before the Parliamentary Committee. From the Blue Book, No. 5745-46.

MR. HENRY DROOP RICHMOND.

Question. "With regard to the action of salicylic acid on enzymes, is your opinion of that subject based upon what you have read or upon what you have done? You say that salicylic acid has an action on the enzymes."

Answer. "I think it is chiefly based upon what I have read. I have also found that the action of certain enzymes, diastase for instance, is stopped by salicylic acid."

The first part of this testimony is indeed very like that of many others. Nearly every opposed authority, when called upon to express his opinion as to the action of salicylic acid on enzymes, almost unhesitatingly states that it is *his opinion* that this preservative retards, or prevents the digestion of food in the stomach and when pressed as to the basis of his opinion, like every one of his predecessors, he states that he has read it. Probably the author of the book also read it as a quotation from some author in the middle of the last century.

Digestive processes were unknown up to the time when Theodore Schwann in 1836 discovered that the gastric juice contained pepsin, and it was only three years previous that Payen and Perzos discovered diastase in malt extract. All literature previous to the discovery of enzymes and organized ferments made no distinction between them.

This idea has been carried down in various scientific works ever since, and it is no very uncommon thing to read extracts (which contain the essence of this fallacy), published within the last few years by authorities who have never given this subject any investigation.

Fermentation is a term very greatly misinterpreted; it is repeatedly treated as a process which is brought about by bacteria, yeasts, and molds, and likewise by the enzymes of digestion. This is a great error, and finds expression in such productions as the following:

North Dakota Agricultural Experiment Station. Bulletin 53. Page 119. In the Minnesota Dairy and Food Commissioner's Report for 1901 this statement appears—preservatives "are used solely to prevent fermentation and since the processes of digestion are fermentation processes, the chemical preservatives must work an injury." And also,

In Bulletin 100 of the Kentucky Agricultural Experiment Station, a well-known officer of the State Food Commissioners' Association says: "The strong paralyzant power claimed for antiseptics is sufficient to condemn their use in foods, for a substance which can preserve perishable foods under any conditions, and for any

length of time, will also affect the delicate digestive ferments of the stomach," and he quotes a government authority in these words: "There is no preservative which paralyzes the ferments which create decay, that does not at the same time paralyze to the same extent, the ferments that produce digestion. . . . The very fact that any substance preserves food from decay shows that it is not fit to enter the stomach."

I was much surprised that the authority mentioned in Bulletin 100 by the writer of that article should have made such a statement, so when in New York later I asked the gentlemen, who is a member of the Committee on Food Standards, if he had been quoted correctly, and he stated that he had never authorized that statement. It is, therefore, up to our Kentucky friend to verify his quotation.

So far as I am aware this statement did not appear in print until Bulletin 100 brought it out, and it has been copied and changed to fit any argument opposed to preservatives by the daily press and the authorities in many states. In the light of modern research it is ridiculous. Any investigator could prove its falsity in a few hours' experiment. Every physiologist knows that hydrochloric acid is present in the stomach and is absolutely necessary in the digestive process. Every one knows that it is an antiseptic, and it is frequently combined with such disinfectants as bichlorid of mercury to increase their antiseptic properties, which it does, from 50 to 100 per cent. It will absolutely prevent the multiplication of bacteria in 0.2 per cent solutions, which is the amount found in the normal stomach during digestion. Yet if we were to accept the statement quoted in Bulletin 100, we would expect to have our digestive apparatus completely paralyzed after every meal.

The bile acids are antiseptics, principally taurocholic acid, which is nearly as powerful as salicylic acid, so affirmed by the following authorities: Lindenberger (Bulletin de la Societe imp. des naturalistes de Moscow, 1884); also Bunge's Physiological and Pathological Chemistry, second edition, pages 184 and 185, quotes as authority Maly and Enrich Monatshefte (Chemistry, Vol. IV, page 89); also Gley and Lambling (Revised biology du Nord de la France, Vol. I, 1886.)

After every meal, when the food reaches the duodenum, the bile flows into it carrying the antiseptic taurocholic acid, and notwithstanding the statement that antiseptics paralyze the ferments, the digestion is stimulated instead. We are almost ready to turn the argument quoted by our Kentucky writer and make it apply in the opposite way, thus, "the ferments that produce digestion are stimulated by antiseptics in the same proportion that the organisms which produce decay are paralyzed."

Prof. Prescott, of the University of Michigan, is quoted in Bulletin No. 53, page 118, of the North Dakota Agricultural Station, as follows: "A food that is braced against decomposition may be found to be braced against digestion." I am quite sure that the author never intended this to be garbled in this manner by anyone, because the statement has an element of truth in it. Antiseptics are not used to completely brace foods against decomposition; indeed it may be truly said that manufacturers of table condiments such as catsup, Chili-sauce, apple-butter, peach-butter, preserves, jam, jellies, etc., do not use preservatives to completely retard decomposition, but to assist the sugar and the container to arrest fermentation until such foods are consumed. It is well known that any of these manufactured products will spoil within a certain time after the original package is opened. Then again, some foods are chemically changed by high temperatures so that they become indigestible. Even the dark brown crust of bread is thus changed by heat in the baking process. Milk is changed to some extent in pasteurization and complete sterilization. Then again the author quoted uses the words "may be" because he undoubtedly recognized the fact that his statement could not apply in all cases and be made to do duty as an argument against preservatives in general.

In the report of 1892, of the Committee on Interstate and Foreign Commerce of the House of Representatives, on the pure food bills, page 395, it is stated that "Prof. Mitchell, of Wisconsin, considers any active antiseptic necessarily deleterious to health. It retards the processes of the stomach, stopping the working of the normal enzymes or ferments."

In the light of our experiments with salicylic acid and pepsin, and from what we know of hydrochloric acid and taurocholic acid, such statements as that of Prof. Mitchell are ridiculous. Here again we have an opinion given with no data or experiments to prove it true, and that opinion is on record to be quoted as an established fact for years to come. Now here is another opinion which is directly opposite to the statement made by Prof. Mitchell. The professor of Physiological Chemistry at Yale University declares that "Antiseptics do not interfere with digestion" (page 396 of the same report). This opinion, coming as it does, from such high authority, and based upon personal investigation, as we know it to be, throws a shadow on the professor from Wisconsin. Here is another opposing opinion by Dr. E. H. Starling, Fellow of the Royal College of Physicians and a Fellow of the Royal Society. (Blue Book Report of the Parliamentary Committee No. 6941, page 243.)

Question. "What have you to tell us as regards salicylic acid?"

Answer. "Salicylic acid is certainly harmful, less, however, than formalin. In an acid medium, that is to say, the medium in which the stomach digestion goes on, it acts as an antiseptic, but in the stomach where it is acting as an antiseptic, it also prevents the action of the gastric juice and stops digestion in the stomach. Clinically, of course, one knows that the use of salicylic acid, especially in the free state, is apt to cause symptoms of gastric dyspepsia, pain in the stomach, and stoppage of gastric digestion, etc."

This authority states in the beginning of his testimony, that he had made physiological experiments with salicylic acid, but he does not state what they were, nor how they were made. He does not state whether he used small doses or whether he administered abnormal quantities. We know that his testimony as to the stoppage of digestive processes in the stomach is absolutely false, if he used less than one part to 500, because we have demonstrated by actual experiment that digestive processes are a little delayed but not entirely stopped, as he stated the case. In quantities less than one to 500 the delay is not noticeable and in either case the delay is no greater than that caused by many substances, such as common salt, coffee, tea, etc., which enter the stomach in a mixed diet.

Clinically, salicylic acid is a fine remedy for fermentation, caused by living organisms in the stomach. It not only destroys the bacteria, but actually assists the gastric juices in the digestion. Two cases of this nature have come under my personal notice, so this would indicate that the testimony of Dr. Starling was not as scientific as it should have been. He goes on to say, "When the food gets down to the intestines, where the medium is alkaline, and where it is attacked by the pancreatic juice, salicylic acid does not disturb digestion, and there, of course, it does not act as an antiseptic." Late investigation has shown that salicylic acid is decomposed in the stomach into carbonic acid and phenol, which are carried off by the urine.

Prof. William Henry Cornfield was called before the committee and testified as follows: "I have had very little practical experience on the results of the internal administration of salicylic acid, but I have studied the effects of it as they have been observed and published by others." This statement was all right, and he probably said just what all the other witnesses should have said, but he goes on with his testimony just as if he had made a deep physiological and chemical research. Note his answer to question 5067 (page 177). "Suppose a person were taking a small quantity of salicylic acid day by day, is it certain he could get rid of the whole of that quantity in each day?" Answer—"I do not *think* it is." Question No. 5077 (page 177): "Might there be a tendency

to accumulation?" "Yes; I think there is evidence that there is a tendency to accumulation with that drug."

As we stated before, there is no evidence to show anything of the sort, and it is well known that salicylic acid and some other preservatives are almost entirely excreted in the urine and perspiration. Like all chemical work this test shows a very slight amount which cannot be accounted for, but when the decrepancies are taken into consideration in other analytical work, it is a fair presumption that the unaccounted per cent. is a chemical decrepancy due to inaccuracy in analysis.

I have been impressed while reading over a large amount of testimony offered on various preservatives, that only rarely is a specific quantity named. We have all along presumed that no greater amount of preservative should be used for physiological research work than it is possible for anyone to actually consume daily in food. This is not more than one part in 500 in some few articles, and not more than one part in 2000 in others. It is hardly likely that anyone would eat as much as one part of salicylic acid to 1000 parts of general food and fluids. This proportion would be very high indeed, and I do not believe that such an amount of preservatives is ever used continuously by anyone.

Any physician could say that certain amount of preservatives would prove harmful, and the same thing could be said of fire—a certain amount of heat is absolutely necessary, but we cannot reason that because a certain amount of heat will burn us that we should not use heat—such reasoning would be absurd. Heat is good, but too much of it must certainly stop digestion; sugar, salt, coffee, tea, etc., are all good in certain amounts, but too much of any will stop digestion, therefore any testimony which does not specify a fixed amount of preservative that is harmful has no value as evidence, except in Pennsylvania.

We have gathered considerable evidence, as our readers have found, to show that the statements by various authorities on the harmful action of salicylic acid on gastric digestion are not strictly in accordance with facts. We have yet to speak of the argument advanced by several investigators to the effect that the diastatic enzyme, ptyalin of the saliva, is stopped by such preservatives as salicylic acid and benzoic acid.

In order to answer this argument, put up by Prof. H. A. Weber and others, we must outline the whole digestive process and consider the nature of each enzyme, and then learn how wisely Nature has planned the whole apparatus so that disturbances in one quarter do not necessarily disturb the whole process.

The ptyalin or diastatic enzyme of the saliva is secreted in an alkaline fluid, and its process is carried on in an alkaline fluid.

(Gamgee's Physiological Chemistry of the Animal Body 1893, pages 23-27).

The pepsin or albumen digesting enzyme is acid and requires the presence of an acid; even small quantities of alkaline solution render it inert. (Langley, Journal of Physiology, III. page 246).

The trypsin or albumen digesting enzyme of the pancreas "works best in a weak alkaline solution." (Ferments and Their Action, page 39, Oppenheimer.) Weak acid solutions do not greatly interfere with it, but any solution containing as much acid as the gastric juice (estimated at 0.2 per cent.) would stop this enzyme.

Now this is certain, therefore, that any acid or alkaline taken either as a food or in food, must have some influence on one or more of these enzymes. It is the acid in such preservatives as salicylic and benzoic acids that has a retarding influence on the diastatic enzyme of the saliva. If this action is to be considered harmful by the opponents of preservatives, and if this is to be considered as a reason for prohibiting preservatives, then the same objection must be filed against every acid food. Lemonade, phosphated drinks, acid fruits of all kinds, have a far greater influence on the ptyalin than all the added preservatives one could take in food, as it is now prepared.

The pepsin of the gastric juice is retarded by all alcoholic drinks, beer, wine, etc. "Beer, even when containing less than three per cent. of alcohol, has a strong restrictive action, and this is not to be ascribed to the hops, since wine has the same effect." (Ferments and Their Action, page 96; also Buchner, Arch. f. klin. Med., XXIV. 537.)

Tea and coffee also retard the action of the pepsin on coagulated egg albumen, demonstrated in National Canners' Laboratory, June "Index," 1904. Frazer also authorizes this conclusion. (Journal of Anatomy and Physiology, XXXI., page 469.)

The amount of salt usually taken in a single meal retards peptic digestion as much, if not more, than all the preservatives taken in food during the same meal. (National Canners' Laboratory Report for May "Index," 1904.

Now note the following: "The gall normally precipitates the pepsin, but when this function is absent—e. g., in fistula—the pepsin penetrates into the intestines and destroys the trypsin to a more or less pronounced extent, and thereby the digestion of the albumen is checked." (Ferments and Their action, page 109.)

The arguments based on the ground that preservatives should not be used in food, because they restrict or retard the digesting enzymes, grow very weak in the face of the facts presented here. If our physical economy were so delicately constructed and so easily upset, we would all be constant sufferers from indigestion. Nature has provided a way to neutralize acids and salts and means of throwing off unnecessary and undesirable substances.

Since it has become known among scientists that the preservatives commonly used in food products are largely excreted by the kidneys, a claim has been set up that the extra amount of work which is forced upon these already over-worked (?) organs, will necessarily lay the foundation for kidney diseases of various kinds. In Bulletin No. 100 of the Kentucky Agricultural Experiment Station, page 101, speaking of preservatives, the writer says that "they are eliminated by the kidneys and that such elimination gives rise to various forms of kidney trouble."

This statement is made without any quotation from medical literature to sustain it, and the reason is obvious, since no such statement is thus made, so far as I am able to find. A question was once asked of a noted physician, "Within the last few years Bright's disease has increased, and is it not possible that preservatives in food products might be one of the principal causes?" He answered that he did not think so, because there are several other causes at work, which had such direct responsibility for kidney diseases, that it was hardly likely that preservatives commonly used in food products were in any way contributory; that more kidney diseases were directly caused through invasion by micro-organisms, such as the gonococcus and bacterium pyocyaneus, also streptococcus and staphylococcus aureus et al; and since such increase of gonorrhoeal infections has been reported by the various medical societies, kidney diseases may be traced in many cases directly to this malady. It is reasonable to suppose that preservatives would have an antiseptic influence on micro-organisms which invade the kidneys, and as antiseptics they would prove beneficial, as indeed they are, and are prescribed in medicinal doses for that very purpose. There is a theory that antiseptics taken in food continuously must exert an irritating influence on the kidneys, and eventually must cause abnormal changes, followed by the invasion of micro-organisms.

The kidneys are endowed by nature with the power to dissolve all kinds of irritating and poisonous substances from the blood stream, and afterwards to expel them through the urine, so it is not a fair presumption to suppose that they are injured because certain substances are supposed to have irritating properties. We should naturally expect to find some evidence of irritation on the mucous membrane of the stomach prior to any injury of the kidneys. In cases of Bright's and kindred diseases, the post mortems often reveal the fact that the stomach is normal in every way, and this would seem to prove that hyperplastic processes were caused through invasion by bacteria.

There is very little medical testimony to prove that diseases of the kidneys are due to overwork in eliminating substances which

pass unchanged through the body. One writer has mentioned salt as a substance excreted by the kidneys. This chemical passes through the body as sodium chlorid and does not cause any abnormal processes of kidney degeneration. Another writer speaks of water as a substance which passes unchanged through the body and is excreted by the kidneys without any injury to them; therefore, it is not a sound argument that because a substance passes unchanged through the body, it must overwork the kidneys.

There are probably no organs of the human body more admirably capacitated for work than the kidneys. There are two of them, and ordinarily there is very little more than enough work for one; the other is always ready, however, to assist in the elimination of foreign substances from the blood stream. Some experiments have been made with animals to determine working capacity of the kidneys, and it was proven that three-fourths of a kidney could be cut away before any serious consequences could be detected. (Albutt's System of Medicine, Vol. IV., p. 318.)

Americans consume large quantities of nitrogenous foods and an unusual amount of work is forced upon the kidneys in order to expel the nitrogen, but there are a number of cases on record where human beings have lived for many years with only one kidney, which had to do all the work devolving upon these organs. This would indicate that any person having two normal kidneys would not be seriously overworking them by allowing them to expel a very small quantity of such preservatives as salicylic or benzoic acid.

The kidneys must excrete the uric acid from the body, and salicylic acid is helpful in its removal, and for this reason the preservative is given as a remedy for rheumatism. (Practical Therapeutics-Hare, page 341.)

Salicylic acid unites with glycin, forming salicyluric acid; also, benzoic acid unites with glycin, forming hippuric acid, and both of these antiseptics are, therefore, helpful in assisting the kidneys to expel the glycin, or as it is commonly called glycocoll, or amidoacetic acid. (Cushny-Pharmacology and Therapeutics, page 417.)

In the London Lancet of November 25, 1899, page 1,427, Cushny rather opposed salicylic acid, because it was excreted from the body in a form unlike any product of normal urine, but he did not oppose benzoic acid because it was excreted as hippuric acid, which is found in normal urine.

Dr. R. G. Eccles has pointed out the false reasoning in this, however, because:

Salicyluric acid is salicylic acid plus glycin.

Hippuric acid is benzoic acid plus glycin.

In other words, there is very little difference between the two, salicylic acid being simply equivalent to hippuric acid plus water.

"As glycin or glycocollic acid is a precursor of urea, its removal by means of salicylic or benzoic acid, aids the body in getting rid of its waste product." (American Text-book of Physiology, page 981, also Foster's Text-book of Physiology, page 539.)

It would seem from this that preservatives such as these are beneficial rather than detrimental. Normal kidneys cannot be injured by them, and since they are antiseptic by nature, it is fair to presume that any one who is suffering with kidneys diseased by invading bacteria must be benefitted by the preservatives which have inhibitory influence upon bacterial life, providing that such antiseptics are not taken in doses large enough to be irritating or to cause cellular metabolism.

CHAPTER VIII.

Preservatives—Continued

Experiments With Preservatives and Other Substances to Determine Their Effect on Peptic Digestion. Physiological and Pathological Research Work With Animals Fed on Salicylic and Benzoic Acids. Post Mortems. Conclusions.

EXPERIMENTS WITH PEPSIN.

A number of flasks were prepared, using the formula for testing the activity of pepsin given in the Pharmacopoea. A series of experiments were made as follows:

Flask No. I.—

12½ grams hard boiled white of egg.
125 c. c. pure water.
0.2 per cent. hydrochloric acid.
0.005 gram of pepsin.

Flask No. II.

Just the same as No. 1 with the addition of ¼ gram of salicylic acid dissolved in water.

Flask No. III.—

Just the same as No. 1 with the addition of ¼ gram of common salt.

Flask No. IV.—

Just the same as No. 1 with the addition of one loopful of Bacillus Vulgaris and one loopful of a bacillus found in corn.

All these flasks were kept for six hours in the incubator at a temperature of 150° F. and agitated frequently.

No. 1 showed only a few flakes, and all the rest had a small quantity of egg still undigested. After two or more hours these were just like No. 1.

This proves that salicylic acid in very strong solutions does not impede digestion more than other substances consumed in a mixed diet, common salt retarding fully as much as the acid. Ordinary bacteria taken at meal time have the same retarding influence, although the amount of hydrochloric acid used prevented them from propogating.

In order to test the retarding influence of various substances in comparison with preservatives, a number of flasks were prepared as follows:

Flask No. I—

125 cubic centimeters of water.
12.5 grams of hard boiled white of egg, finely divided.
0.005 gram of pure pepsin.
0.2 per cent hydrochloric acid.

Flask No. II—

Just the same as No. I with the addition of 0.25 gram of preservative commonly used in food products. Benzoic Acid.

Flask No. III—

Just the same as No. I with the addition of 0.25 gram of ground coffee.

Flask No. IV—

Just the same as No. I with the addition of 0.25 gram of green tea.

Flask No. V—

Just the same as No. I with the addition of 0.25 c. c. of absolute alcohol.

Flask No. VI—

Just the same as No. I with the addition of 0.10 gram of salicylic acid, 0.10 gram of coffee and 0.10 gram of tea.

These were placed in the incubator and kept for six hours at 105° F. and frequently shaken. The results are most interesting.

In flask No. 5 the coagulated egg albumen dissolved first; that in No. I was second; No. VI was third with just a few flakes undissolved; No. II was fourth; No. IV was not complete; and No. III was decidedly retarded, fully half of the egg remained undigested. No. VI was the most interesting from the fact that it contained three substances which are claimed to retard digestion. This flask contained coffee, tea and salicylic acid and complete digestion of the egg albumen followed the typical experiment represented in flask No. I. It can be explained in but one way—i. e., that the small quantity of salicylic acid used acted as a stimulant.

Flask No. V, containing the absolute alcohol, was the first to complete the digestion of the egg albumen, and we must conclude that a very small per cent of alcohol stimulates digestion, while we know that when the per cent is increased to 3 it greatly retards digestion. (Buchner, Arch. f. Klin. Med. XXIX-537.)

We find that salicylic acid in minute quantities stimulates digestion. This is true of all acids. (Oppenheimer—Ferments and Their Action, page 97.)

In the proportion of 1 to 500 it retards digestion slightly, just about as much as common salt, and in larger amounts it will possibly interfere with digestion. The conclusion to be drawn is that the amount of any substance is a very important factor in determining its action on digestive processes. Certain quantities may be positively beneficial, while increased amounts may be injurious.

FEEDING PRESERVATIVES TO ANIMALS, POST MORTEM, AND PATHOLOGICAL ANALYSES OF INTERNAL ORGANS.

One of the arguments advanced by the opponents of food preservatives is the opinion of many eminent physicians that the delicate mucous membrane of the stomach will become irritated and inflamed, and thereby be injured to a greater or less degree, if preservatives, and particularly salicylic acid, be taken continuously in food and drink. This objection, although founded as we believe on nothing but mere speculation, would seem to be a very strong one indeed. We all know how easily the stomach responds to the action of chemicals and even to the influence of the mind. The cells of the mucous membrane are very sensitive to any influence not exactly in accord with normal conditions. Even the state of the nerves of the body has an influence; nauseating odors and disgusting sights are influences which completely upset the stomach. Anger, passion, joy, sorrow and other influences of the mind act upon the cells of the mucous membrane, and for a time the stomach cannot perform its work normally. We all know that certain drugs, medicines and improper food will cause stomach derangement for a certain time; some medicines completely upset the normal condition, others stimulate the action of the enzymes, and still others cause inflammation of the stomach. Some physicians say, then, it is a very probable conclusion that preservatives will irritate the stomach, and this argument above all others is made to do duty as a strong reason why these chemicals should not be permitted in foods. There are a number of cases on record where persons have purposely taken various quantities of one preservative or another in daily doses, and we have very favorable testimony to the effect that no evil results followed, although in many cases abnormal amounts were taken. When abnormal quantities of salicylic acid are taken the warning is sounded by a ringing or buzzing sensation in the ears, similar to the effects felt after taking quinine in large doses. It is a remarkable fact that no discomfort has been felt from taking stated quantities of salicylic acid below the amount which will cause a ringing sensation in the ears.

Diligent search has been made for the names of any persons who would give evidence that they had experienced any ill effects from stated doses of salicylic acid under the amount that will cause the ringing sensation or fullness in the head. No such amount of this preservative is ever used in food products. There are only three or four products which require more than 1.1000 of any preservative, and this amount is very small in proportion to the full meal. Such condiments as require a preservative are used only sparingly at meal time; as a rule they are used to improve other food or make it more palatable. No one would make an entire meal

Plate 70. Four Guinea Pigs selected as controls.

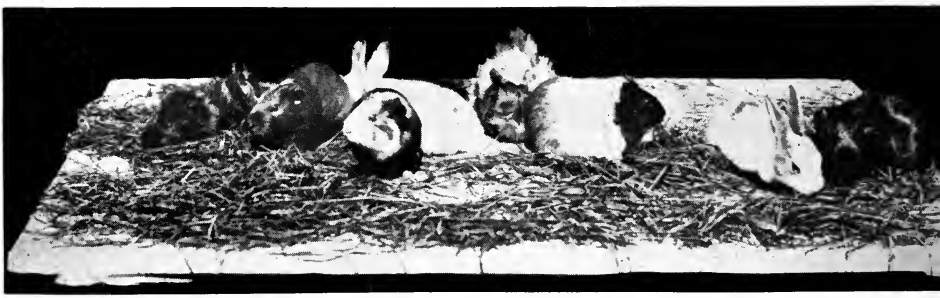


Plate 71. Six Guinea Pigs and Two Rabbits.
Whose food was saturated with salicylic acid.

of tomato catsup or chili-sauce; only a small quantity is taken on cold meat, on oysters or in soup. Very few persons would use these condiments at more than one, possibly two meals per day, consequently the amount of such preservative as is used to keep them in an unfermented condition would be very small indeed.

We have heard several authorities say that if preservatives were used only in such food as we have specified there would possibly be very little objection to them, but the claim is made that perhaps nearly all the prepared food of various kinds contained pre-

servatives, and in such amounts would prove dangerous and poisonous to the human organism; also that if preservatives were permitted in one kind of food it would be difficult to draw the line and prevent their employment in all foods. Then the argument follows that in such amounts they would injure the mucous membrane of the stomach and other internal organs.

It is easy to assume that certain effects will follow certain causes, but it is a very difficult matter to disprove the assumption, so when a large number of physicians assert positively that the mucous membrane of the stomach will be injured by salicylic acid in the amounts ordinarily used in food products, the amount of work required to overthrow such an assumption is large, and the technique employed in conducting the experiments is difficult, requiring special skill and a thorough knowledge of pathology. To this end a series of experiments were conducted with animals, and Dr. W. H. Ingram, professor of pathology in the West Penn Medical College, and Dr. R. G. Burns, pathologist and bacteriologist for the City of Allegheny, Pa., undertook the pathological part of the work. A number of guinea pigs were fed a weighed amount of salicylic acid daily. The acid used was the synthetical product, that which has been used so extensively in food. 7½ milligrams of the acid were mixed in a breakfast cereal with milk, which amount was fed daily to each pig. This amount was found too large because the animals would not eat it unless forced to do so by hunger, so the amount was cut down to 5 milligrams. The only food taken by the pigs outside of the prescribed diet was grass, which was fed to them liberally. Comparing the weight of a guinea pig with that of an average man, which is as 1 to 100 or 1 to 150, the same proportion of acid would be from 7½ to 10 grains daily for a man, and this amount of preservative would be sufficient to inhibit the growth of micro-organisms in 16 to 24 ounces of solid food in proportion of 1 to 1000. This amount of preservative is far in excess of the amount taken in food daily by any person, but it represents an extreme case, but nevertheless one which might be possible. It is possible that in rare cases a person might consume such a quantity of certain foods and drinks that he would take as much as 10 grains or more in a single day, but this would not be continued daily. Physicians prescribe sodium salicylate in 30 grain doses three times daily for rheumatism. In this there would be about 22 grains of pure salicylic acid in each dose, consequently 60 grains daily for a limited time would not endanger a person. Therefore, if any person should happen to take more than 10 grains at a single meal or in one day, he would not suffer any special inconvenience.

We are certain that the experiment conducted with the guinea pigs on a basis of 5 milligrams of salicylic acid daily is sufficiently broad to cover all cases.

The experiments were conducted with guinea pigs in preference to any other animals for several reasons. There are no animals which live on a diet exactly the same as a human, and in selecting animals we must not select any such as the dog or cat which eat food partially decomposed, because the stomachs of such animals are not as sensative to poisons as the human. The stomach of the guinea pig is sensitive to poisons and cannot tolerate certain foods. Even milk is not properly digested unless mixed with cereal. Another reason for selecting guinea pigs is that they are herbivorous. They are accustomed to a vegetable diet, and since nearly all the food products, in which a preservative is used are made from fruits and vegetable, these animals seem to be well suited for the experiments. They are cheap, easily handled and are not very large, so that every advantage may be claimed for their selection.

The first series includes two pigs fed on 5 milligrams of salicylic acid mixed with cereal daily, and one pig fed just the same amount of cereal, but no acid (this one was used as a control). By having controls we are able to determine any physiological differences, and the post mortem comparisons are quite valuable and necessary.

When these experiments were begun the pigs were not full grown and none of them showed any signs of pregnancy. One of this series was a female and pregnant, which became apparent toward the end of the period from the increased weight and shape of the animal, but it was decided to learn if any pathological changes could be found in such cases, therefore she was submitted for analysis.

SERIES NO. (1)

The weights of these three guinea-pigs were taken every other day, the results of which are here appended in a table.

WEIGHT OF ANIMALS IN OUNCES.

DATE	MAY 14	MAY 16	MAY 18	MAY 20	MAY 22	MAY 24	MAY 26	MAY 28	MAY 30	JUNE 2	JUNE 5	JUNE 7	JUNE 9	JUNE 11	JUNE 13	JUNE 15
Brown Female Pregnant	18¼	18¼	18¼	18½	19½	19¾	20¼	20¾	20¼	20¼	21½	21¾	21¾	22	22¼	24
Brown Male	17½	17⅝	18	18¼	19	19	19¼	19¼	18¾	18¾	19½	19½	19½	19¾	19¾	20½
White (Male Control)	x	x	x	x	17	17¾	18½	18	17½	17¾	18¾	18¾	19¼	19½	19¾	20¼

The table increase in weight for all three guinea-pigs up to May 30, when for some unaccountable reason they all lost and this

was not regained until five days later. This decrease in weight was true of the control as well as of the others, indicating that the salicylic acid was not the cause. In the thirty days guinea-pig No. (1) had gained 5½ ounces; No. (2) had gained 5 ounces; No. (3) the control, gained only 3¼ ounces in the 24 days.

All three animals were then given to Dr. W. H. Ingram for pathological examination. He is an eminent authority on pathology and the following is his report:

DR. INGRAM'S REPORT.

Pittsburg, Pa., July 15, 1904.

I here append analyses of guinea pigs, June 15, 1904, marked Pigs 1, 2 and 3, *Series 1.*

I. GENERAL APPEARANCE.

All these are well nourished animals and very active.

Pig No. 1, female, pregnant, near full term. Color, red and white.

Pig No. 2, male. Color, black and brown.

Pig No. 3, male, marked "Control." Color, white and yellow.

II. POST-MORTEM. APPEARANCES.

All three animals were killed at the same time. Examination shows as follows: Animals still warm.

I. Pig No. 1. Subcutaneous fat normal. Axillary and inguinal lymph glands normal. Muscle normal.

1. Abdominal Cavity—

1. Stomach partially filled with grass and macerated food. Emptied, and musculature contracted normally. No change in color.

2. Intestine—Normal. Various portions contain faecal matter of character found in parts.

3. Liver—Normal in position and size.

4. Gall Bladder—Normal; partially filled with bile.

5. Pancreas—Normal in size and position.

6. Spleen—Normal in size and position.

7. Kidneys—Normal in size and position.

8. Suprarenals—Normal in size and position.

9. Uterus—Contains three pigs. Estimated about one week short of full term. Pigs normal. Placenta normal.

10. Bladder—Partially emptied. Urine contained, normal.

11. Mesentary and Glands—Normal.

2. Thoracic Cavity—

1. Lungs—No pathological changes either in position or size.
2. Heart—Normal in size and position. Right side partially distended with blood. Muscle color normal.

II. Pig No. 2—

1. Subcutaneous fat normal in amount. Axillary and lymph glands normal in size.

1. Abdominal Cavity—

1. Stomach—Partially filled with well macerated and partially digested food, principally grass. Emptied, and muscle wall contracted normally. Color not altered.
2. Intestine—No changes from normal in size or contents.
3. Liver—Normal in size and position.
4. Gall Bladder—Distended with bile.
5. Pancreas—Normal in size and position.
6. Spleen—Normal in position, color and size.
7. Kidneys—Normal in position, color and size.
8. Suprarenals—Normal in position, color and size.
9. Testes—Normal in position, color and size.
10. Bladder—Empty and firmly contracted.
11. Mesentery and Glands—Show no pathological changes.

2. Thoracic Cavity.

1. Lungs—No changes appreciable.
2. Heart—In position, color and size normal. Right side partially filled with blood.

III. Pig No. 3—

This animal, marked "Control No. 1" was a normal, healthy male, and careful examination failed to show any pathological processes. All organs were inspected as in Pigs Nos. 1 and 2.

III. MICROSCOPIC EXAMINATION.

1. Technique.

The organs from these animals were placed in 4 per cent solution of Formaldehyde for fixation. This step was completed while the parts were still warm. The stomach and intestines were spread out flat. Other solid organs were cut into slices about 2 m. m. thick. After remaining in this fixing fluid for about 40 hours, they were placed in running water for 24 hours, and then in 80 per cent alcohol for preservation.

Parts of each solid organ, measuring 2 c. m. long by 1 c. m. wide and 1 m. m. thick were then placed in alcohols of 90 per cent, 95 per cent and "100 per cent" ("absolute alcohol," 99.8 per cent —Squibb) for 24 hours for each per cent. The wall of the stomach was cut in sections having about the same surface area, and including all the coats. The sections of the stomach were placed in the ascending alcohols with those from the solid organs.

From the alcohols they were immersed in a mixture of equal parts of 99.8 per cent alcohol and ether for a period of 24 hours.

The imbedding was in celloidin. Two solutions were used. One, thin celloidin, in which the sections remained 48 hours, and the

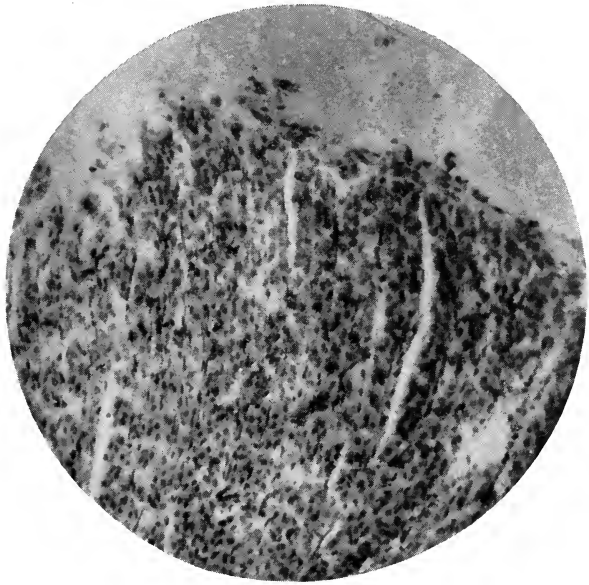


Plate 72

Photomicrograph showing glands of the mucous membrane of the stomach, cardiac end. There is no evidence of degenerative changes. Guinea pig No. 1. Spencey 2 m.m. objective. 1X compensating eyepiece.

second, thick celloidin (practically a saturated solution in alcohol and ether), in which they remained 48 hours. They were next placed in "wood fiber" blocks, hardened for 24 hours in 80 per cent alcohol, and cut on a "Minot Perusian Microtome," 2 to 3-micra thick.

Staining was produced by the Hematoxyln and Eosine method, and sections were mounted in Balsam.

All sections stained easily, with sharp differentiation.

2. Results of Examination—

I. Pig No. 1—

1. Stomach. (Section G. P. 527 and 526.)

Mucosa. (1). Surface epithelium—Does not show any pathological changes. Nuclei are sharply stained and normal in size and position. Cell protoplasm does not show any evidence of degenerative changes, staining evenly.

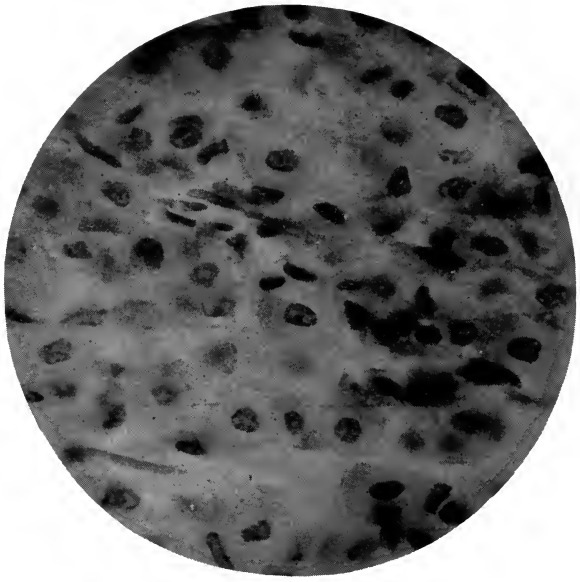


Plate 73

Photomicrograph showing mucous membrane of the stomach of guinea pig No. 1. Cardiac end. The cells are all normal and show the nuclei plainly. Photographed through the microscope, using a 2 m.m. objective and 6X compensating eyepiece.

(2) Glands—These are normal in shape and arrangement. No changes can be seen in the lumen or the cells forming the gland. The central cells show no changes, staining evenly. In the cardiac glands the Parietal Cells are very sharply defined, nuclei staining evenly, and the protoplasm showing no evidences of degeneration. The interglandular connective tissue does not show any tendency to leukocytic or round cell infiltration, nor does it show any other evidence of either hyperplasia or inflammation. The vessels of the mucosa are normal. The lenticular glands are also normal.

No changes either of an inflammatory or degenerative character can be seen in the other coats.

2. Spleen. (Section G. P. 528.)

This organ shows no lesions. The capsule is not, in any part examined, thickened, or shows signs of inflammation. Trabeculae also normal. Malpighian bodies show no evidences of any degenerative changes. Pigment in sinuses and cells not affected. Blood vessel walls normal.

3. Heart. (Section G. P. 524.)

Plate 74

Photomicrograph—Suprarenal and adjoining adipose tissue, showing capsule, cortex and part of medulla. 2 m.m. objective and 1X compensating eyepiece.

Plate 75

Photomicrograph—Spleen, Showing malpighian bodies. 16 m.m. objective and 6X compensating eyepiece.

(1) Endocardium—No evidence of degeneration nor of inflammation.

(2) Myocardium—Muscle Fibers—Nuclei normal, striations distinct. No changes in intercellular substance. The interstitial connective tissue normal in amount. No evidences of any inflammatory or degenerative process can be seen.

(3). Pericardium—This does not show any pathological process.

Plate 76

Photomicrograph showing the involuntary muscles of the heart of guinea pig No. 2. The striations are well marked and the cells show no degenerative changes. 2 m.m. objective with 12X compensating eyepiece.

4. Kidneys. (Sections G. P. 531^1 and 531^2.)

Both kidneys were examined. The malpighian bodies do not show, either in the capsule of Bowman, nor in the glomerulus, and evidences either of an inflammatory or degenerative character. The same may be also said of the tubule, from the neck to the termination. The lumen of the tubule is at no place filled with unnatural products.

The interstitial connective tissue is also normal. Blood vessels show no changes of any type.

5. Suprarenals. (Section G. P. 532.)

This shows no evidence of any pathological character either in the capsule, connective tissue, cortex or medullae.

6. Pancreas. (Section G. P. 529.)

This organ is also normal. The bodies of Langerhaus do not show any evidences of pathological processes. The cells of the glands stain clearly, evenly and are of normal size and shape. Various sections show the same normal character.

1. Placenta and Uterus. (Section G. P. 530.)

Examination shows a normal placenta. The uterine wall, as well as the placental attachment, show no disease conditions whatever.

II. Pig No. 2—

This pig shows characters in no way differing excepting those of sex from those described. Taking the organs in the same order they show the following:

Plate 77

Photomicrograph of a section of the pancreas of guinea pig No. 1, showing lobules, centro-acinar and secretory cells. There is no evidence of pathological changes. Magnified by 2 m.m. objective and 1X compensating eyepiece.

1. Stomach. (Sections 536 and 537.)

Surface epithelium normal, nuclei and protoplasm staining evenly and typically.

The glands do not present any alterations in shape or position. The "central" and "parietal" cells staining in characteristic manner.

The "lenticular glands" present same characters as those of stomach of Pig No. 1.

There are no evidences of any pathological changes in the interstitial connective tissue.

The same may be said of the remaining coats of this organ.

2. Spleen. (Section G. P. 539.)

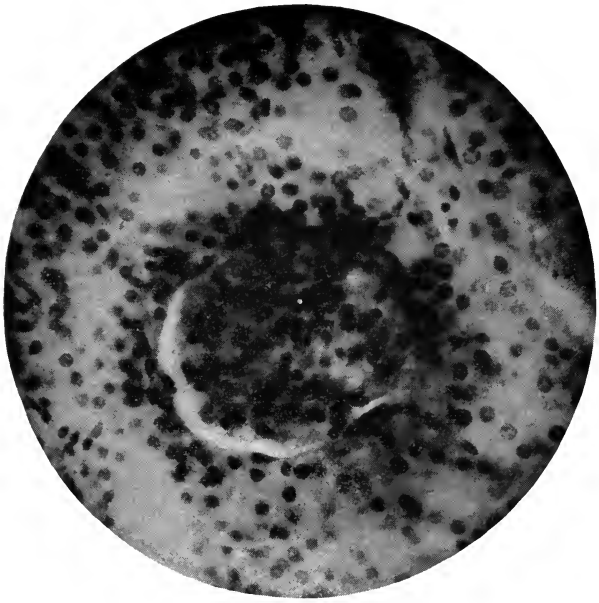


Plate 78

Photomicrograph—Kidney showing malpighian body and convoluted tubules of guinea pig No. 2. All cells normal. 2 m.m. objective and 6X compensating eyepiece.

Plate 79

Photomicrograph of section of kidney of guinea pig No. 2, showing malpighian bodies and convoluted tubules. No evidence of degenerative changes. Cells normal. 16 m.m. objective and 6X compensating eyepiece.

Malpighian bodies normal in size and number. No alterations in the walls of the blood vessels. Capsule and trabeculae show no evidences of inflammatory or degenerative changes.

3. Heart. (Section G. P. 533.).

(1) Endocardium normal.

(2) Myocardium, shows no changes either in the vessels, muscle fibers nor the interstitial connective tissues.

(3) Pericardium, same as the endocardium.

4. Kidneys. (Section G. P. 537.).

These organs present characters similar to those of Pig No. 1. The Malpighian bodies, tubules, interstitial connective tissues and vessels are normal.

5. Suprarenals. (Section G. P. 538.)

Both these organs are normal, presenting no evidences of disease.

6. Pancreas. (Section G. P. 540.)

This presents no character differing from that of Pig No 1, being normal.

Pig No. 3. Control.

All organs of this animal were examined, but as it was a normal, healthy animal, they presented no characters of interest in this connection. By comparing organs from this animal with those of No. 1 and No. 2, no differences could be seen, either in structure or staining reactions.

CONCLUSION.

The conclusion to be drawn from these sections, after careful comparisons, would be that all three animals were in a healthy condition, and that whatever diets may have been administered, they had no effect on the various organs, so far as any structural alterations were concerned. I could not detect any evidence, either of a degenerative, inflammatory, necrotic or hyperplastic process.

W. H. INGRAM.

SERIES NO. II.

The second series of experiments to determine the effect of preservatives on animals was begun on May 14. Three guinea pigs were fed 5 milligrams of salicylic acid daily. The preservative was mixed with a well-known breakfast cereal. Two were fed with 5 milligrams of benzoate of sodium daily, and other animals were fed the same food as these, but no preservatives. These were controls.

There were three females in the series and we did not know in the beginning that they were pregnant, but as they increased in weight so rapidly, it became apparent; therefore, on June 11 we dis-

WEIGHTS OF ANIMALS IN OUNCES. Series No. 11.

DATE	MAY 14	16	18	20	22	24	26	28	30	JUNE 2	5	7	9	11	13	15	17	19	21	23	25	27	29	JULY 1	3	5	7	9	11	13	15	17	18
GUINEA PIG.—No. 1. *Male.* Black Shaggy Coat. Fed 5 Milligrams Salicylic Acid daily	18¼	18¼	18½	18½	19¼	19½	20	19½	19	19	19½	19¾	19¾	19¾	19¾	20½	19¾	19¼	19½	19½	19½	19¾	20	19¾	20	20½	21	22¼	22¼	22	21	21½	22
GUINEA PIG.—No. 2. *Male.* White, black hind quarters. Fed 5 Milligrams Salicylic Acid daily	24½	24½	24¾	25	25¼	25½	26	26¼	25½	26	26¼	26½	26¾	27	27¼	27½	27½	28	28¼	28¼	28½	28	28¼	28¾	28¾	30½	30½	31	31¼	29¾	29½	29½	30¼
GUINEA PIG.—No. 3. *Female.* White and brown. Fed 5 Milligrams Salicylic Acid daily	18½	20¼	20¼	20¾	22¾	23¾	24½	24¾	23½	24½	25¾	26	27	.....	.....	.....	.....	.....	.....	3 Pigs born	.....	.....	.....	.....	20¼	20¼	20¾	20	19½	19¼	19 ¼	19½	19¾
GUINEA PIG.—No. 4. *Female.* Brown, red and white. Fed 5 Millegrams Benzoate Sodium daily	21¾	22¼	22¼	22½	25	26	27	27	26½	27¼	28¾	29½	30	.....	.....	3 Pigs born	.....	.....	.....	.....	.....	.....	.....	.....	21¼	21¼	21¼	20¼	21	19½	20	21	21½
GUINEA PIG.—No. 5. *Female.* Dark brown and white. Fed 5 Milligrams Benzoate of Sodium daily	17	17¼	17¼	17½	18½	18¾	19½	19	18¼	19¾	20½	21½	21¾	.....	.....	.....	.....	.....	.....	.....	3 Pigs born	.....	.....	.....	21¼	21¼	21¼	21¼	21¼	21¼	20	20½	20½

Total time of Experiment, 65 days. Each animal in that time consumed 325 millegrams of preservatives.

continued taking the weights of these three. June 17-23 and 25 were celebrated by the birth of the young ones. On July we again began taking the weights as before. This we continued to do until July 18, when the five animals were submitted for pathological and histological analysis.

CONDITIONS NOTED DURING FEEDING.

As stated it was not known that the three females were pregnant at the beginning of this experiment, but it was decided after finding out the fact, that it was just as well to go through the ex-

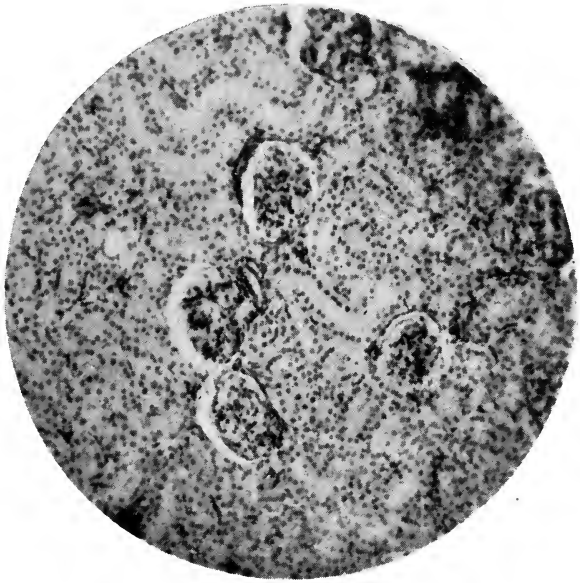


Plate 80

Photomicrograph of Kidney of guinea pig No. 4, showing malpighian bodies and cell arrangement. There is no evidence of inflammatory or degenative changes. Magnified 150 diameters.

periments under natural conditions. The very fact that the three females gave birth to young ones within the term is interesting. The young pigs were immediately given adult doses of preservatives as soon as they were able to take food themselves, and the next series will show the result. During the term there was no sign of sickness on the part of these animals; all seemed active, particularly the two males. These were quite vicious. One day we had a terrible rain storm which flooded the quarters where these animals were kept and fed, and rather expected that they might show some signs of sickness, but they did not. This would seem to indicate that the preservatives given had no weakening effect.

The report of Dr. Ingram here appended is very satisfactory.

I here append results of analysis of guinea pigs Nos. 1 to 5, Series 2, sent to my laboratory for pathological examination.

I. POST-MORTEM EXAMINATION.

These animals were all killed at the same time, and "posted" while still warm, in manner similar to those of Series 1.

PIG NO. 1.—MALE.

(Fed 5 milligrams salicylic acid daily for 65 days.)

(a) Black, shaggy coat—a very vicious animal and well developed. Subcutaneous fat, abundant. Lymph glands of inguinal, axillary and cervical regions not enlarged; musculature, normal.

(b) Abdominal Cavity—No gross lesions of any character detected; all organs being normal in size and position. Contents of viscera, normal.

(c) Thoracic Cavity—Lungs distended, normal color; heart and vessels show no changes; no enlarged lymph glands.

PIG NO. 2.—MALE.

(Fed 5 milligrams salicylic acid daily for 65 days.)

6 (a) White, black hindquarters. Very large pig. Vicious and well developed. Subcutaneous fat, normal; musculature, normal. Axillary, inguinal and cervical glands, normal.

(b) Abdominal Cavity—Nothing abnormal, either in position or size detected. Visceral contents, normal.

(c) Thoracic Cavity—Nothing abnormal in color, position or size of lungs, heart or lymphoid tissue.

PIG NO. 3.—FEMALE.

(Fed 5 milligrams salicylic acid daily for 65 days.)

(a) White, brown hind quarters, nose black, well developed, soft coat. Subcutaneous fat normal. Musculature, same. No enlargement of lymph glands, of axillary, inguinal or cervical regions.

(b) Abdominal Cavity—No pathological changes in size, shape or position of organs. Not pregnant. Uterus and ovaries normal. No enlargement of lymphatic structures. Contents of viscera normal.

(3) Thoracic Cavity—Nothing abnormal of any character detected.

PIG NO. 4.

(Fed 5 milligrams benzoate sodium daily for 65 days.)

(a). Brown and red forequarters and face; white hindquarters. Well developed, smooth coat. Subcutaneous fat abundant. Musculature, normal. No enlargement of lymph glands in any part.

(b) Abdominal Cavity—No changes of any character detected. Uterus contains four embryo pigs, 1.5 c. m. long. These are normal.

(c) Thoracic Cavity—Nothing abnormal.

PIG NO. 5.—FEMALE.

(Fed 5 milligrams benzoate of sodium daily for 65 days.).

(a) Dark brown, white face, right cheek black; left red. Well developed animal. Subcutaneous fat abundant. Musculature normal. No enlargement of lymphoid glands.

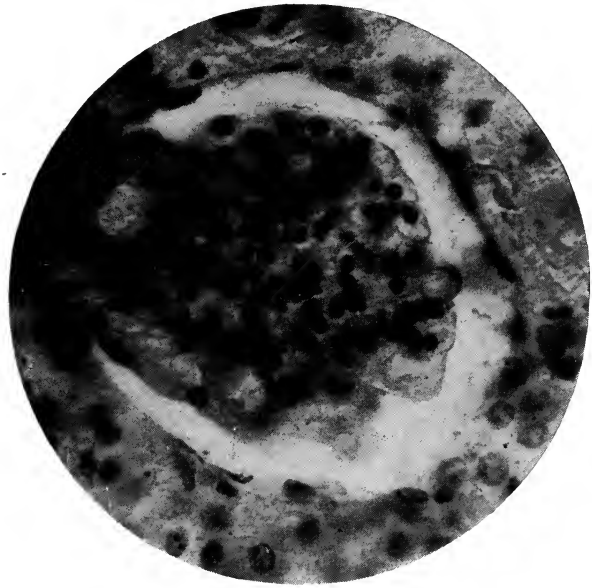


Plate 81

Photomicrograph Kidney, snowing malpighian body and convoluted tubule of guinea pig No. 1. Bowman's capsule and the beginning of the urinary tubule or canal. All cells are normal. Magnified 500 diameters.

(b) Abdominal Cavity—Nothing abnormal detected in size, shape or position. Visceral contents normal.

(c) Thoracic Cavity—No pathological changes detected.

MICROSCOPIC EXAMINATION.

PIG NO. 1.—SERIES NO. 2.

1. Heart—No. 554 normal in all parts, myocardium, pericardium and endocardium.

2. Pancreas—No. 555 normal, no changes in bodies of langerhaus.

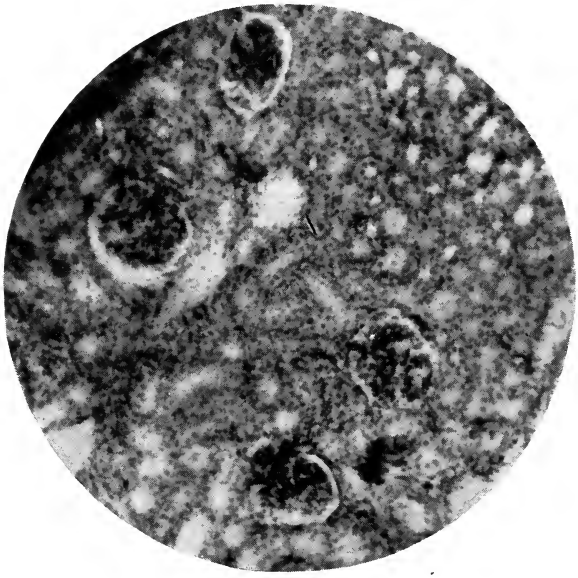


Plate 82

Photomicrograph of sections of kidney of guinea pig No. 5. No degenerative changes either in the capsule to Bowman nor in the glomerulus, The ascending and descending limbs of Henle's loop are normal. The interstitial connective tissue cells are stained with distinct differentiation. Magnified 150 diameters.

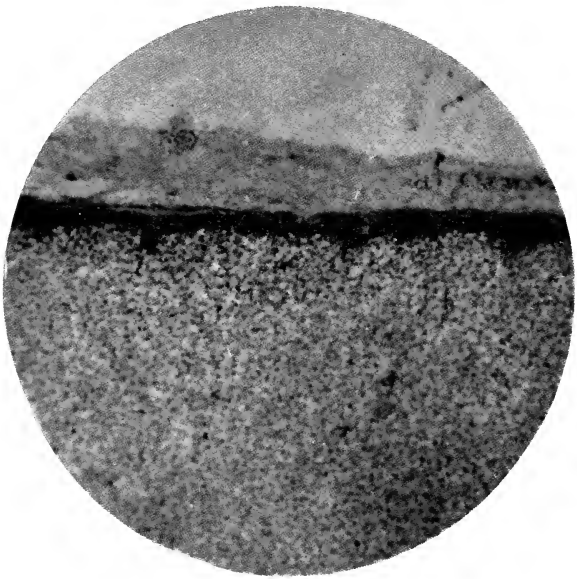


Plate 83

Photomicrograph of Suprarenal and adjoining adipose tissue showing capsule, cortex and part of medulla. The cells are normal. No pathological changes. From a section of guinea pig No. 4 which was fed 5 milligrams of benzoate of sodium daily for two months. Magnified 100 diameters.

3. Kidneys—No. 556 normal. No pathological changes.
4. Testicle—No. 559, normal. Spermatogenesis very active.
5. Suprarenals—No. 556, normal.
6. Liver—No. 558, normal.
7. Stomach—No. 557, normal. No changes in either glands of mucosa or in any parts.
8. Spleen—No. 556 normal. Malipghian bodies show no proliferation or degeneration.
9. Lung—No. 553, normal.

PIG NO. 2.—SERIES NO. 2.

1. Heart—No. 574, normal.
2. Lungs—No. 573, normal.
3. Stomach—No. 578, normal.
4. Pancreas—No. 759, normal.
5. Liver—No. 583, normal.
6. Liver and gall—bladder—No. 582, normal.
7. Kidneys—No. 581, normal.
8. Suprarenals—No. 581, normal.
9. Spleen—No. 580, normal.

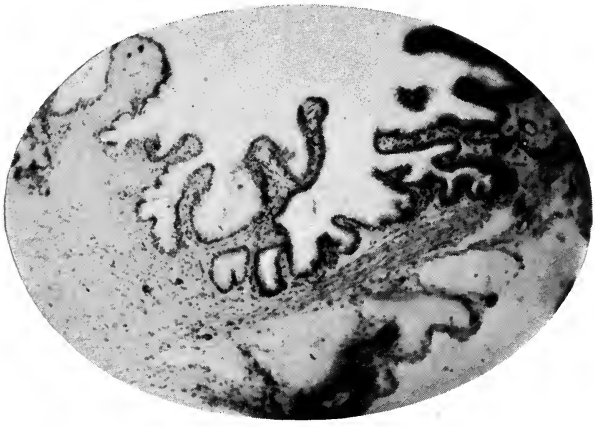


Plate 84

Gall bladder photomicrograph, showing the wall which is composed of the mucus, fibro muscular, subserous and serous coats. There are no degenerative changes shown in the cells of the wall. The glands in the tunica propria of the mucosa are normal. From guinea pig No. 1, series II, which was fed 5 milligrams of salicylic acid daily for two months. Magnified 100 diameters.

PIG NO. 3.—SERIES NO. 2.

1. Heart—No. 562, normal.
2. Lungs—No. 563, normal.
3. Liver—No. 565, normal.
4. Oesophagus—No. 564, normal.
5. Stomach—No. 568, normal.

6. Kidney—No. 567, normal.
7. Suprarenals—No. 567, normal.
8. Liver—No. 566, normal.
9. Spleen—No. 569, normal.
10. Intestine—No. 571, normal.

PIG NO. 4.—SERIES NO. 2.

1. Heart—No. 584, normal.
2. Lungs—No. 583, normal.
3. Liver—No. 587, normal.
4. Spleen—No. 590, normal.
5. Pancreas—No. 588, normal.
6. Stomach—No. 589, normal.
7. Kidneys—No. 586, normal.
8. Suprarenals—No. 587, normal.
9. Placenta and uterus—No. 592, normal.
10. Foetus—No. 593, normal.

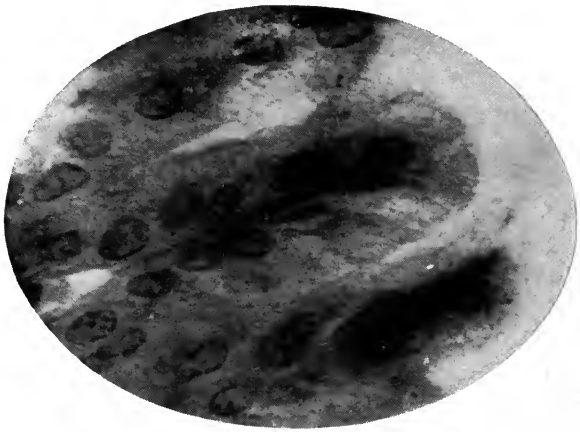


Plate 85

Photomicrograph showing glands of the mucous membrane of the stomach, cardiac end. There is no evidence of degenerative changes. Guinea pig No. 1. The cells are all beautifully arranged, and show no neicrotic processes nor injury from the daily dose of preservatives. Magnified 750 diameters.

PIG NO. 5—SERIES NO. 2.

1. Heart—No. 542, normal.
2. Lungs—No. 543, normal.
3. Liver—No. 544, normal.
4. Spleen—No. 546, normal.
5. Pancreas—No. 547, normal.
6. Stomach—No. 548, normal.
7. Uterus—No. 549, normal.
8. Fallopian tubes—No. 549, normal.

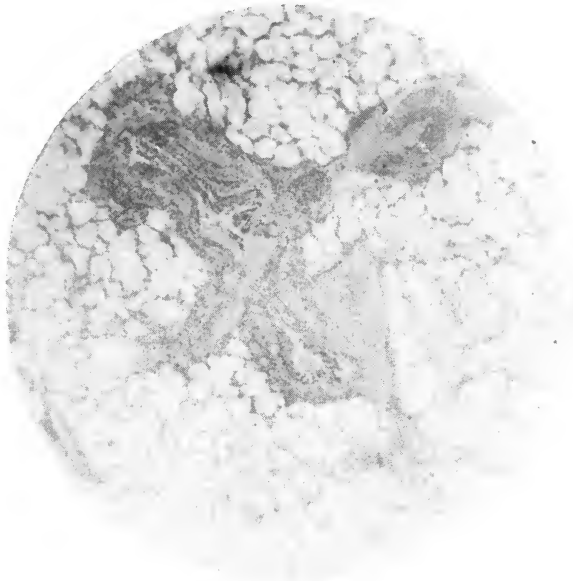


Plate 86

Photomicrograph of the lung of guinea pig No. 1, showing bronchus with convoluted mucosa. The clear spaces are air passages and infundi bula. The notches in the walls of the clear spaces are the air vesicles Conditions normal. Magnified 50 diameters.

Plate 87

Photomicrograph showing glands of the mucous membrane of the stomach, cardiac end. The cells are normal in size, and there is no evidence of inflammation or degenerative changes. Section made from stomach of guinea pig No. 5, which was fed on 5 milligrams of benzoate of sodium daily for two months. Magnified 500 diameters.

9. Kidneys—No. 551, normal.
10. Suprarenals—No. 551, normal.

The same technique was employed as in Series 1.

SERIES NO. 3.

The third series of experiments was conducted with animals which were fed on preservatives from the time they were able to take food themselves—from babyhood up to maturity. Two of these were fed 5 milligrams of salicylic acid daily, and two were fed 5 milligrams of benzoate of sodium daily. During the whole course of feeding, they showed no signs of sickness, but on the contrary, seemed more active and healthy than the controls, born about the same time.

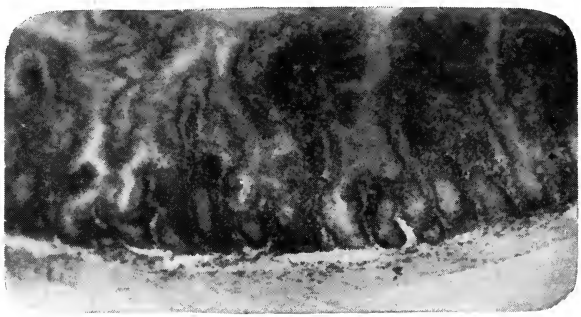


Plate 88

Photomicrograph of the intestine of guinea pig No. 2, showing the crescentic valve-like folds of the mucosa and the villi, also the tubular glands called follicles or crypts of Lieberkuhn. Running along the base is the muscularis mucosae. Conditions normal. Magnified 50 diameters.

Considering the age and size of these guinea pigs, and the amount of the preservatives fed to them, we regard this as a crucial test, and from a therapeutical standpoint, might be sufficient evidence that these preservatives are harmless. Three of these animals were born on July 15, their mother being the fourth animal in Series No. 2. The other one was born on July 23, its mother being the third animal in Series No. 2. Just one week after these baby pigs were born, they were put on the preservative diet, in the same proportions as were administered to the full grown animals in the other series.

On September 1 we took them to the laboratory for pathological and histological analysis.

A study of the table will show that all of these little pigs gained steadily in weight, if anything they gained more in proportion than the other young ones born about the same time, and which were not fed on any preservatives. The following is Dr. Ingram's report:

WEIGHT OF GUINEA PIGS IN OUNCES. Series No. 3.

DATE.	JULY 22	24	26	28	30	1	3	5	7	9	AUGUST 11	13	15	17	19	21	23	25	27	29	SEPT. 31	1	MOTHER.
GUINEA PIG. — No. 1. *Male.* Black face, white fore-quarters. Fed 5 milligrams Salicylic Acid daily.....	7½	8½	8½	9	9½	10	10	10¾	11¼	11¼	11¼	11½	12	12¼	12½	13	13	13	13¼	13½	13¾	14	Guinea Pig No. 4. Series II.
GUINEA PIG. — No. 2. *Male.* Red and Brown face. Fed 5 milligrams Benzoate of Sodium daily	.....	....	.....	.....	6½	7	7½	7¼	8	8¼	8	9	9	9	9¼	9¼	9½	9¾	10	10¼	10½	10¾	Guinea Pig No. 3. Series II.
GUINEA PIG. — No. 3. *Male.* White Stripe in face. Fed 5 milligrams Salicylic Acid daily...	7¼	8¼	8¼	8½	9½	9¾	10¼	10½	11¼	11¼	11	12	12	12	12½	12½	12¾	12¾	13	13¼	13½	14	Guinea Pig No. 4. Series II.
GUINEA PIG. — No. 4. *Male.* White and Black face. Fed 5 milligrams Benzoate of Sodium daily	5¼	5¾	5¾	6¼	7	7¼	7¾	8	8½	8½	8½	9½	9½	9½	9¾	10	10	10¼	10½	10¾	11	11½	Guinea Pig No. 4. Series II.

Feeding term for Guinea Pigs, except No. 2, was 42 days. No. 2 was 35 days.

I here append reports of examination of guinea pigs Nos. 1 to 4, Series 3. These four pigs were healthy and very active.

The animals were killed at the same time, and in similar manners as Series No. 1 and No. 2.

(1) POST-MORTEM APPEARANCES.

GUINEA PIG NO. 1, SERIES 3.

(a) Male, black face, white forequarters, red hindquarters. Subcutaneous fat, normal. No enlargement of the lymph glands. Musculature normal.

(b) Thoracic cavity—No lesions appreciable of any of the organs.

(c) Abdominal cavity—All organs normal in position and size.

Plate 89

Photomicrograph showing glands of the mucous membrane of the stomach of guinea pig No. 1. Cardiac end cells are normal in size and there is no evidence of inflammation or degenerative changes. Magnified 500 diameters.

GUINEA PIG NO. 2, SERIES 3.

(a) Male, right side of the face brown, left side red, body red, right foot front white. Subcutaneous fat normal. No glandular enlargement. Musculature normal.

(b) Thoracic cavity—No change in position, size, etc., of the organs of this cavity.

(c) Abdominal cavity—Organs normal in size and position.

GUINEA PIG NO. 3, SERIES 3.

(a) Male. Right forequarters and face red. Left forequarters and face brown. Both hindquarters white. White stripe in the median line of the face. Subcutaneous fat and musculature normal. No glandular enlargements.

(b) Thoracic cavity—Contents normal in size, etc.

(c) Abdominal cavity—Organs normal in position, size and contents.

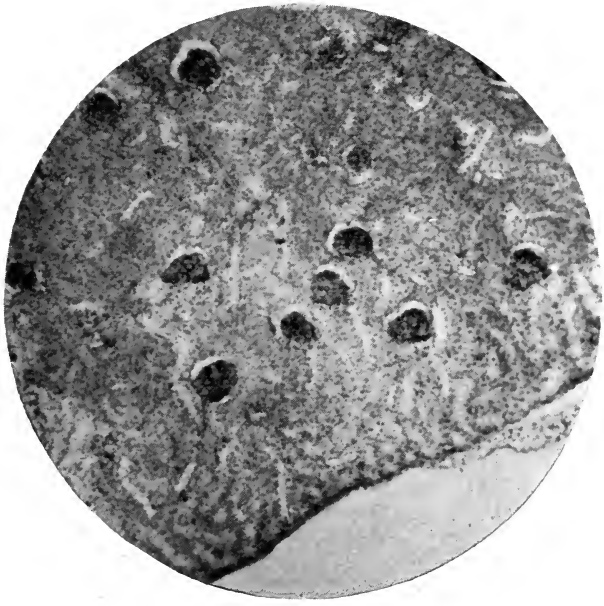


Plate 90

Photomicrograph of a section of Guinea Pig No. 2. No degenerative changes either in the capsule o Bowman or in the glomerulus. The ascending and descending limbs Heule's loops are normal in size and appearance. The collecting tubules are normal. The interstitial connective tissue cells are stained with distinct differentiation. Magnified 100 diameters.

GUINEA PIG NO. 4, SERIES 3.

(a) Male. Right side of face black, left white, right forequarters black, left white; left hindquarters black, right hindquarters white and black. Subcutaneous fat and musculature normal. No enlargement of the lymph glands.

(b) Thoracic cavity—Nothing abnormal in position and other indications.

(c) Abdominal cavity—Normal in contents.

(2) Microscopic examination.

(1) Guinea Pig No. 1, Series 3. (Fed on 5 milligrams salicylic acid daily).

(1) Lungs—Guinea pig, section No. 598, normal.

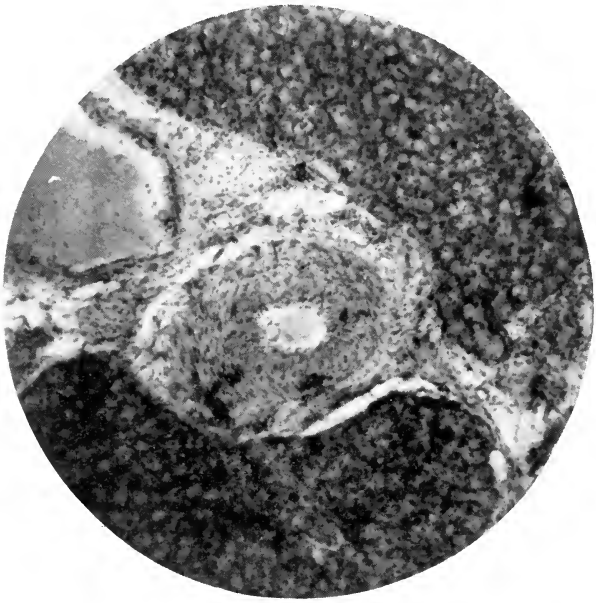


Plate 91

Photomicrograph of the Pancreas of guinea pig No. 2. The bodies of Langerhaus and gland cells are normal in size and form. Magnified 200 diameters.

Plate 92

Photomicrograph of the small intestine of guinea pig No. 3, showing the villi glands of Lieberkuhn, Lymph nodules, Muscularis mucosae, Submucosa and Muscularis. No changes. Normal. Magnified 100 diameters.

(2) Heart—Guinea pig, section No. 599, normal.
(3) Spleen—Guinea pig, section No. 600, normal.
(4) Pancreas—Guinea pig, section No. 601, normal.
(5) Stomach—Guinea pig, section No. 602, normal. Contents, partly digested food.
(6) Liver and gall bladder—Guinea pig, section No. 603, normal (both).
(7) Kidneys and suprarenals—Guinea pig, section No. 604, normal (both).
(8) Small intestine—Guinea pig, section No. 605, normal.
(2) Guinea pig No. 2, Series 3. (Fed on 5 milligrams of sodium benzoate daily.)

Plate 93

Photomicrograph showing glands of the mucous membrane of the stomach. Normal. From guinea pig No. 4. Magnified 100 diameters.

(1) Lungs—Guinea pig, section No. 606, normal.
(2) Heart—Guinea pig, section No. 607, normal.
(3) Spleen—Guinea pig, section No. 608, normal.
(4) Pancreas—Guinea pig, section No. 609, normal.
(5) Stomach—Guinea pig, section No. 610, normal. Contents same as No. 602.
(6) Liver and gall bladder—Guinea pig, section No. 611, normal (both).
(7) Liver (second section)—Guinea pig, section No. 612, normal.

(8) Kidneys and suprarenals—Guinea pig, section No. 613, normal (both).

(3) Guinea pig No. 3, Series 3 (Fed on 5 milligrams of salicylic acid daily).

(1) Heart—Guinea pig, section No. 614, normal.

(2) Lungs—Guinea pig, section No. 615, normal.

(3) Spleen—Guinea pig, section No. 616, normal.

(4) Pancreas—Guinea pig, section No. 617, normal.

(5) Stomach—Guinea pig, section No. 618, normal. Contents same as No. 602.

(6) Kidneys and Suprarenals—Guinea pig, section No. 619, both normal.

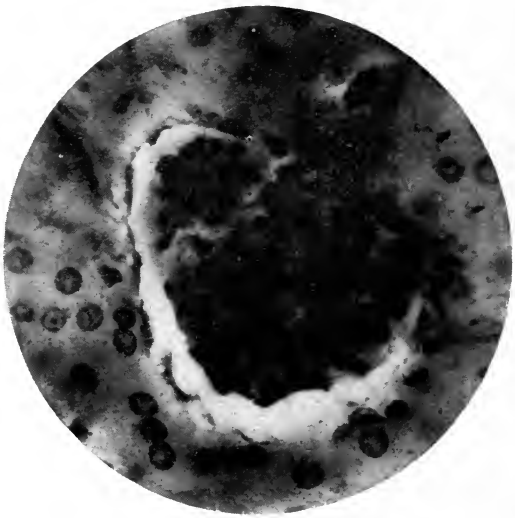


Plate 94

Photomicrograph of kidney, showing malpighian body and convoluted tubule of guinea pig No. 3. Bowman's capsule and the beginning of the urinary tubule or canal. All cells are normal. Magnified 450 diameters.

(7) Liver and Gall Bladder—Guinea pig, section No. 620, both normal.

(4) Guinea pig, No. 4, Series 3. (Fed on 5 milligrams of sodium benzoate daily.)

(1) Heart—Guinea pig, section No. 621, normal.

(2) Lungs—Guinea pig, section No. 622, normal.

(3) Spleen—Guinea pig, section No. 623, normal.

(4) Pancreas—Guinea pig, section No. 624, normal.

(5) Stomach—Guinea pig, section No. 625, normal. (Contents same as No. 602.)

(6) Kidneys and Suprarenals—Guinea pig, section No. 626, both normal.

(7) Liver and Gall Bladder—Guinea pig, section No. 627, both normal.

CONCLUSION.

The technique being the same in these three series, a uniform result was thus obtained. From these analyses of this series, the same conclusion must be arrived at as in the series No. (1) and (2), that is, that the conditions these guinea pigs were subject to, had no effect, so far as producing lesions of any organs, appreciable by pathological methods.

W. H. INGRAM.

SERIES (4). RESULT FROM FEEDING RABBITS ON PRESERVATIVES.

It has been stated by famous pharmacologists, that it is good therapeutics to base an opinion as to the effects of a drug on the results obtained by feeding in stated quantities to animals. If there were no physiological ill effects noticed and if the internal organs showed no hyperplastic, pathological or degenerative changes, it was a fair assumption that the drug would have no harmful or injurious effect upon the human organism. This is indeed the method employed to determine the character of all known substances, particularly at the time they are first discovered.

We have completed the fourth series of such experiment with salicylic and benzoic acids and this series has some interesting features. On May 14 we received several very young rabbits along with our guinea pigs, and selected two for experimental purposes. These appeared in the photograph published in the May issue of the "Index" and are reprinted here.

The young rabbits which we intended for controls did not get along well and soon died, but the two kept for the experiment grew rapidly and were never sick a single day during the whole term of feeding. They were always active and playful and we became so much attached to them that we disliked to kill them for the pathological analysis. During the months of August and September we gave the 5 milligrams of benzoate of sodium daily, in addition to their daily dose of 5 milligrams of salicylic acid. They seemed to relish their food very much and would always come running to us every time we approached their cage. They were always hungry and I believe we might have given them double the quantity of preservatives without injuring them in the least. During the term of feeding they never seemed drowsy and I often wondered when they obtained enough sleep, for on moonlight nights I could see them running around very lively.

On May 14 the white bunny weighed 11¾ ounces and the grey weighed 7¾ ounces—they were mere babies. From that date upto August 1 we fed them 5 milligrams of salicylic acid

daily and they weighed at that time 35 and 34 ounces respectively. After that we administered the two preservatives as previously mentioned and at the end of the term they weighed 49½ and 43 ounces respectively. They were then killed for pathological and histological analyses by Doctor R. G. Burns, a noted pathologist, and the bacteriologist for the city of Allegheny, Pa. The following is his report:

DOCTOR BURNS' REPORT.

————, ————, Nov. 15, 1904.

I herewith submit reports of examination of rabbits:

These two rabbits were very active and healthy.

The rabbits were killed at the same time and in similar manner by chloroform. The technique was as follows. The organs of

Plate 95

Photomicrograph showing glands of the gastric mucous membrane. There is no evidence of degenerative changes. The cells, with their nuclei, are beautifully stained with Haematoxylin and Eosine. The Parietal, smooth muscle, and chief cells are plainly visible. Magnified 500 diameters. Rabbit No. 1.

these animals were placed in a 4 per cent solution of formaldehyde, the several organs were cut into slices two millimeters thick. After remaining in this fixing solution for 36 hours they were placed in running water for 24 hours and then in 60 per cent alcohol, then 80, and finally in absolute alcohol.

From the alcohol they were placed in equal parts of absolute alcohol and ether for 36 hours.

The embedding was in celloidin, two solutions, one thin, in which they remained for 48 hours, the other thick. They were next

DATES.	5–14	5–16	5–18	5–20	5–22	5–24	5–27	5–29		6–1	6–3	6–5	6–7	6–9	6–11
WHITE RABBIT.	11¾ ozs.	11¾	12¼	13½	1–1	1–3½	1–6	1–6¾		1–7¼	1–8	1–10¼	1–11¼	1–11½	1–12
DATES.	7–11	7–13	7–15	7–17	7–19	7–21	7–23	7–25	9–27	7–29	7–31	8–2	8–4	8–6	–8
Male.	2–1¾	2–1¾	2–2	2–2	2–2¼	2–2¼	2–2¼	2–2½	2–2½	2–2¾	2–3	2–3	2–3	2–3	2–3½
DATES.	9–7	9–9	9–11	9–13	9–15	9–17	9–19	9–21		9–23	9–25	9–27	9–29	9–31	10–2
	2–5½	2–5¾	2–6	2–6½	2–6½	2–6¾	2–7	2–7		2–7½	2–7½	2–8¾	2–9	2–10	2–10½
DATES.	5–14	5–16	5–18	5–20	5–22	5–24	5–27	5–29		6–1	6–3	6–5	6–7	6–9	6–11
	7¾	8½	11	1¼	13½	15¾	1–1½	1–2¾		1–3¼	1–4	1–6¼	1–6½	1–6½	1–6½
DATES.	7–11	7–13	7–15	7–17	7–19	7–21	7–23	7–25		7–27	7–29	7–31	8–2	8–4	8–6
GREY RABBIT.	2–1	2–1	2–1	2–1¼	2–1¼	2–1	2–1¼	2–1½		2–1½	2–1½	2–1¾	2–2	2–2	2–2¼
DATES.	9–5	9–7	9–9	9–11	9–13	9–15	9–17	9–19		9–21	9–23	9–25	9–27	9–29	9–31
Male.	2–3¾	2–4	2–4	2–4½	2–5	2–5	2–5½	2–6		2–6¾	2–7	2–6¾	2–7	2–7¼	2–7½
DATES.	6–13	6–15	6–17	6–19	6–21	6–23	6–25	6–27		6–29	7–1	7–3	7–5	7–7	7–9
WHITE RABBIT.	1–12½ ozs.	1–13¾	–15	–15½	1–15¾	1–15¾	1–15¼	2–0		2–0½	2–1	2–1	2–1½	2–1¾	2–1¾
DATES.	8–10	8–12	8–14	8–16	8–18	8–20	8–22	8–24		8–26	8–28	8–30	9–1	9–3	9–5
Male.	2–3¾	2–3¾	2–3¾	2–4¼	2–4	2–1¼	2–4½	2–4½		2–4¾	2–5¼	2–5½	2–5¼	2–5¼	2–5½
DATES.	10–4	10–6	10–8	10–10	10–12	10–14									
	2–11¼	2–12½	2–14	2–14½	2–15¾	3–1½									
DATES.	6–13	6–15	6–17	6–19	6–21	6–23	6–25	6–27		6–29	7–1	7–3	7–5	7–7	7–9
	1–7	1–8¾	1–9	1–10¾	1–13¼	1–13½	1–14¼	1–14½		1–15	1–15½	1–15¾	2–0	2–0¼	2–1
DATES.	8–8	8–10	8–12	8–14	8–16	8–18	8–20	8–22		8–24	8–26	8–28	8–30	9–1	9–3
GREY RABBIT.	2–2	2–2¼	2–2¼	2–2½	2–2½	2–2¾	2–3	2–2¾		2–3	2–3½	2–3½	2–3¼	2–3¼	2–3½
DATES.	10–2	10–4	10–6	10–8	10–10	10–12	10–14								
Male.	2–8	2–8½	2–9	2–8¾	2–9¼	2–9½	2–11								

TABLE OF WEIGHTS.—Animals fed on 5 to 10 milligrams of preservatives daily from May 14 to Oct. 14—Five months.

placed upon wood blocks and cut with Stucket's microtome. Stained with Haemoloxyln and Eosin, mounted in Canada balsam; sections stained easily.

POST-MORTEM APPEARANCES.

RABBIT NO. I.

(a) Grey color, no enlargement of lymph glands, muscular and subcutaneous fat normal.

(b) Thoracic cavity—Organs normal as to position and size.

(c) Abdominal cavity—Organs normal as to position and size, stomach contents partially digested.

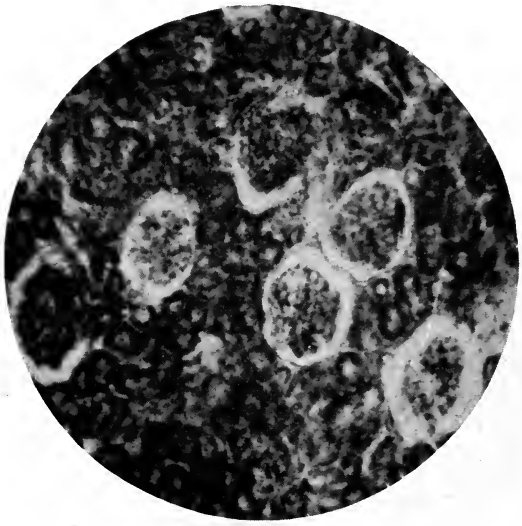


Plate 96

Photomicrograph section of kidney of Rabbit No. 1, which had been fed on preservatives for five months. The malpighan bodies are normal in size, number and position. The tubules of both organs are normal. There are no degenerative changes. The cells are stained clearly. Magnified 250 diameters.

MICROSCOPIC EXAMINATION.

RABBIT NO. I.

1. Lungs normal.
2. Heart normal.
3. Spleen normal.
4. Pancreas normal.
5. Stomach normal.
6. Liver normal.
7. Kidneys normal.
8. Small intestines normal.

POST-MORTEM.

RABBIT NO. 2.—WHITE COLOR.

Organs of thoracic and abdominal cavities normal as to position and size. Lymph glands, muscular and subcutaneous fat normal, stomach contents partially digested.

MICROSCOPIC EXAMINATION.

RABBIT NO. 2.

1. Lungs normal.
2. Heart normal.
3. Spleen normal.

Plate 97

Photomicrograph of gastric mucous membrane of Rabbit No. 2, which had been fed on preservatives for five months. There is no evidence of necrotic hyperplastic, degenerative of inflammatory changes. The glands and cells are normal. Magnified 200 diameters.

4. Pancreas normal.
5. Stomach normal.
6. Liver normal.
7. Kidneys normal.
8. Small intestines normal.

Microscopic slides of which are herewith submitted.

Very truly,

R. G. BURNS, M. D.

Dr. Burns was not present at any time during the feeding term of these animals and was not familiar with the preservative experiment and did not know on what diets the animals had been fed, therefore his analyses are entirely independent of the feeding, and he was not influenced by any knowledge of the facts and his results as are reported just as he found them.

We examined the slides carefully under various magnifying powers and here append our findings.

Slide of the stomach of white rabbit.—The stomach wall including the mucosa, submucosa, muscularis, and serosa, appeared

Plate 98

Photomicrograph of a Malpighan body of the kidney of Rabbit No. 2. The convoluted tubules, Bowman's capsule, Glomerulus, are all normal. The cells, with their nuclei, are well stained with Haematoxylin and eosine. No degenerative changes. Magnified 500 diameters.

perfect in every respect; there were no lesions or anything that would indicate injury. The fundus glands were stained perfectly, showing the parietal cells with nuclei clearly stained; the smooth muscle cells and the chief cells seemed to be properly arranged. The pyloric glands were normal in size and position; the epithelium of the surface, the tunica propria, solitary follicles were all normal. The slide of the gray rabbit's stomach presented no different appearance.

Slide of the kidneys from the white rabbit.—These sections suffered somewhat in mounting. The glomeruli were somewhat disarranged, but there were no disease processes. The convoluted tub-

ules and their cells were all normal, likewise Bowman's capsules. The cells in the glomeruli were all distinctly stained with the nuclei standing out prominently. The blood vessels and urinary tubules were normal in appearance. The ascending and descending arms of Henle's loops are plainly visible, and are normal in appearance. The convoluted tubules of the first order are normal in size, number and position. The general appearance of the kidney consisting of the tunica albuginea, medullary rays, convoluted tubules both of the first and second order, cortex, medulla, veins and arteries was that of a healthy animal and showed no evidence of degenerative processes.

The slide from the gray rabbit had no different appearance so far as we were able to determine.

Since these two organs, viz., the stomach and the kidneys, are the ones most likely to show the effects of any improper diets, we have not made any more extended microscopical examination of the other organs and simply refer the reader to Dr. Burns' report.

The results obtained by the pathological analyses of the four series of animals are most gratifying. We are inclined to believe that all the professional opinions expressed as to the harmful effects of salicylic and benzoic acids in the amounts ordinarily employed as preservatives in food products are unwarranted by the facts. We certainly do not believe them and from what we have observed in these experiments we are inclined to take the other side. We have not been prejudiced, we have been seeking to learn the truth, and we have learned that some of the most respected professional men in this country have been expressing opinions concerning preservatives without making personal investigation. This is lamentable, because it casts a shadow on the integrity of the men who stand out so boldly as opponents of preservatives. Take for instance the statement by a well known champion of the opposition, "that because preservatives are anti-ferments and therefore stop processes of decay, in just that proportion will they interfere with the ferments of digestion." Nothing could be farther from the truth, yet this same error and misconception is embodied in the very first resolution offered to the International Pure Food Congress by the committee on preservatives. It reads: "Whereas, etc., be it resolved (1), That this congress does not approve of the use of preservatives or antiseptics other than those above named (salt, sugar, vinegar and wood-smoke), which, to be effective, must destroy or paralyze all fermentative organisms. They induce a condition which must be more or less unfavorable to digestion and they are therefore to this extent hurtful." This resolution is signed by the following persons: H. W. Wiley, J. H. Shepard, V. L. Price, E. F. Ladd, Julius Hortyett, William Berkely and Richard Fischer.

There is not a single particle of truth in this resolution. Every one of the substances mentioned as being non-injurious have fully as much harmful effect on digestion as the well known preservatives, salicylic and benzoic acids, weight for weight. Some of them will retard digestion more than these, vinegar and creosote from smoke, for example. Hydrochloric acid, one of the most powerful anti-ferments known, is absolutely essential to digestion—digestion by pepsin cannot proceed without hydrochloric acid; how then can the resolution be true? Some of these men, as learned as they are in some directions, do not seem to know that fermentation accomplished by bacteria, yeasts and molds is as much different from the digestive processes as ignorance is from knowledge. Just follow out such reasoning—if all anti-ferments exert an injurious effect on the digestive processes, how in the world are we going to eliminate the 0.2 per cent of hydrochloric acid which is poured into the stomach by the glands of the gastric mucous membrane? Because cranberries contain benzoic acid, shall we refrain from eating that delightful sauce with our turkey dinners, simply because a few radicals declare that it interferes with digestion? Any sensible and thinking person knows better than that; every one knows that cranberry sauce assists digestion, and this sauce, as it is prepared for the table, contains benzoic acid in the proportion of 1 to 2,000 and more. Reverse the reasoning: arsenic is a dangerous and deadly poison to man; it will absolutely stop all digestive processes in small doses, but poisonous as it is to mankind, it has very little effect on bacteria, and in the same proportion does not interfere at all with the putrefactive bacteria. *The statisticians inform us that the average length of life has increased FIVE YEARS within the last decade.* To what cause can this felicitous improvement be traced? Some have attempted to answer this question by stating that the science of medicine has made wonderful strides and new remedies have been discovered. Some say that there are better sanitary conditions, and there are better methods of combating diseases by quarantine laws and regulations. Some say that we have better food since the germ theory has become better understood. Let us ask ourselves a few questions.

What is the most frequent cause of death? Bacteria of diseases?

What is the effect of newly discovered remedies? Do they destroy bacteria?

Has the medical science advanced in proportion to the discovery of the bacteria which cause diseases, and the antiseptics used to combat them?

Has the increased amount of chemically preserved food anything to do with the lessening of diseases? Why have they not

shortened life as the opponents of food preservatives claim? Is it possible that preservatives might be the means of preventing the multiplication of disease bacteria in food?

Is it not possible that the increased longevity may be due directly to the increased consumption of chemically preserved food?

Bacteria are the cause of most deaths, and the antiseptics are the means we have at hand for opposing them. Shall we not be very careful how we restrict their usefulness? Is it not a possibility that the pure food experts are attempting to drive out of our markets the very best food ever prepared for the health and happiness of mankind? We merely ask the questions.

CHAPTER IX.

Chemical Antiseptics

Benzoic Acid.—Method of Detection.—Salicylic Acid.—Method of Detection. Formaldehyde.—Method of Detection. Boracic Acid.—Method of Detection.—Miquels Table of Antiseptics and Their Relative Value.

Only a few chemicals have any very great antiseptic value and the number which is used commonly in preserving food is limited to three or four. Of course, there are various substances which have antiseptic value, such as sugar, salt and vinegar, but we have reference only to chemicals which are prepared and sold to the trade for preserving purposes. These are benzoic acid, more commonly used in the form of benzoate of sodium; salicylic acid, boric acid and borax, and formaldehyde.

Benzoic acid ($C_6H_6CO_2$) is made from benzoin by sublimation. It is artificially prepared from tuluol and may be obtained from toluene (C_6H_8C), or naphthalin ($C_{10}H_8$) from hippuric acid.

The English benzoic acid is prepared from certain varieties of Botany Bay gum (gum acroides), and is superior to the German product. The form usually employed as a preservative is benzoate of sodium ($NC_7H_5O_2$) which is made by simply neutralizing benzoic acid with carbonate of soda.

It is white and very light, with odor of benzoin, has a sweetish astringent taste; it is soluble in twenty parts of water and forty-five parts of alcohol. It is used as a medicine for gout, rheumatism, lithaemia and lithaemic gravel, puerperal fever and tuberculosis. As a preservative it is powerful in the proportion of 1 to 909 (Miquel), and prevents the growth of molds, yeasts and nearly all bacteria. It is employed largely in the manufacture of catsups, pulps, sauces, fruit butters, jams, preserves, meats, beer, wines, and in fact, practically takes the place of salicylic acid, which was prohibited by law in the years 1893 and 1896 in the various states. The laws of many states do not permit the employment of this or any other antiseptic, but there is a disposition to be lenient with the manufacturers of tomato products pending the reports of the results of experiments now being made. There will therefore be no action taken against the use of benzoate of sodium in catsup, Chili-sauce, etc., at present, unless the quantities used are in excess; 1-1000 will prob-

ably be allowed until the department at Washington gets more information on the effect of this chemical upon the human body.

Owing to the fact that many manufacturers of food products purchase part of their preserved material which they use in special formulae, it is well for them to be acquainted with official tests employed to ascertain what preservatives, if any, have been used. The manufacturer will be enabled to make these tests for himself if he observes closely each step in the analysis.

DETECTION OF BENZOIC ACID.

Make a chloroform extract of the material to be examined. If liquid, make slightly alkaline with sodium hydroxid, and first strain through flannel, then acidify with 33⅓ per cent sulphuric acid, and add about 10 per cent of the bulk of chloroform or ether.

If the material is of solid nature, dissolve by maceration with water, a little more than equal weight, and proceed as with liquid.

After making chloroform extraction separate this from water by means of a separatory funnel. If the extract be clear, evaporate at a low temperature until a residue is formed. In the event of the solution not being clear, the result may be obtained by using the centrifugal machine (Fig. XI). Take up residue with a small quantity of hot water and make the following three tests for benzoic acid:

First. Sublimation method (Leach). Evaporate an ammoniacal solution of the ether extract to dryness in a large watch-glass by the aid of a gentle heat. Fasten with clips or otherwise a second watch-glass to the first, edge to edge, so as to form a double convex chamber with a cut filter paper between. Place upon a small sand-bath and heat. Benzoic acid, if present, will sublime upon the surface of the upper glass in minute needles, recognizable under the microscope. It may further be tested by determining the melting-point of the crystals, or by treating the residue with ammonia, and after evaporation and solution of the residue, applying the ferric chlorid test.

Second Method. —Make a part of the extract distinctly alkaline with ammonium hydroxid; expel excess of ammonia by evaporation, take up the residue with small quantity of water and add a few drops of a neutral 0.5 per cent solution of ferric chlorid. The reaction will be a brown-colored precipitate of benzoate of iron, if benzoic acid be present.

Third Method.—Evaporate a portion of the original extract and add a small quantity of chemically pure sulphuric acid, then heat until white fumes are given off. All the organic matter is charred and the benzoic acid is converted into sulpho-benzoic acid.

To this add a few crystals of potassium nitrate, and there is formed meta-di-nitro-benzoic acid.

This acid should be cooled and diluted with water, then add ammonia in excess, and then add a drop or two of ammonium sulphid, which converts the nitrocompound into meta-di-amido-benzoic acid, which possesses a red color and shows immediately on the surface of the fluid without stirring.

If any two of these methods give positive reactions it is safe to assume that benzoic acid is present. The methods given may seem on first reading to be slightly complicated, but they are really simple and can be used successfully by any one who exercises care.

The chloroform and ether extraction of the suspected material will show in the final tests even small quantities of benzoic acid.

SALICYLIC ACID.

In 1834 salicylic acid was discovered by Pagensteecher in the flowers of Spiraea Ulmaria, in the form of salicyl aldehyde. This antiseptic is also found in the oil of wintergreen (Gaultheria procumbens), sweet birch (Betula lenta) and other varieties of gaultheria, also in various fruits and vegetable roots. It is obtained synthetically from carbolic acid or phenol. It has one more atom of oxygen than benzoic acid and its symbol is $C_7H_6O_3$. It is also called ortho-oxybenzoic acid. Kolbe patented a process for obtaining it by treating sodium phenol with carbon dioxide gas. Equal parts were evaporated to powder, then heated to 212 degrees F., then a stream of CO_2 was passed over it and temperature raised to 365 degrees F., then to 428 degrees F., then to 482 degrees F., until phenol ceased to distill over the retort. One-half of the phenol remained and formed salicylate of sodium. P. W. Hofman patented a process by which all the phenol was converted into salicylic acid by using superheated steam in the distillation.

Salicylic acid is a snowy white, very light material, comprised of four-sided prism, which crystallize from hot water in fine prismatic needles. It has a sweetish taste and an acrid after taste. It is irritating to the nostrils, causing violent sneezing. It is soluble in 450 parts of water and 2½ parts of alcohol. It is quite soluble in water containing 8 per cent of borax or 10 per cent of sodium phosphate.

Salicylic acid is one of the *benzyl compounds*, of which there are five in common use. All have the peculiarities of the base benzyl. The other four are benzoic acid, benzaldehyde, salol and saccharin, the latter being intensely sweet and is sold under trade names as a substitute for sugar, to which it has no chemical analogy.

Salicylic acid is antiseptic in parts 1 to 1,000 (Miquel), but exerts marked differences on various bacteria. Some common putre-

factive species remain unaffected in the presence of considerable quantities, while others perish, and this accounts for the losses often experienced when salicylic acid was used largely as a preservative of table condiments of various kinds. This chemical came into favor about 1880 and the quantities imported from Germany were enormous. There were considerable quantities manufactured in the United States, but the acid was inferior in antiseptic power to that of the imported. Up to 1894 it continued in favor and was used to preserve every kind of food subject to fermentation and putrefaction. Laws were rapidly passed prohibiting its employment as a food preservative, owing to the statements issued by several authorities that it was harmful and produced heart trouble or might cause death to persons having heart trouble, and so it was replaced by benzoic acid, neutralized by carbonate of sodium and sold as benzoate of sodium.

Salicylic acid is combined with sodium for medicinal purposes and is a valuable remedy for disordered stomach, due to fermentation, also for rheumatism, etc. It is easily detected by the official test when used only in small quantities.

A chloroform or ethereal extract is made, the same as for the benzoic acid test, and the following tests are reliable:

First Method.—Add two or three drops of ferric chlorid to a small quantity of the extract and let them come together slowly. The reaction is a purple or violet color.

Second Method.—Evaporate 0.5cc. of the extract to dryness at a low temperature, and add one drop of nitric acid (C. P.), then make alkaline with a few drops of ammonia. Ammonium picrate is formed, having a yellow color, which may be used to dye a thread of clean wool.

For a crude test the presence of salicylic acid in such food products as catsup, Chili-sauce, etc., may be determined by simply diluting the material with water and using a few drops of ferric chlorid, which produces the purple or violet color. The simplicity of this test was a cause for the rapid discontinuance of salicylic acid as a preservative after the laws were passed prohibiting it in foodstuff.

FORMALDEHYDE.

Formaldehyde is a very powerful antiseptic which has lately come into use, especially as a preservative for milk and some other food substances, also as a disinfectant and deodorizing agent. Formaldehyde is symbolized CH_2O, and is closely allied to formic acid, which acid results from oxidation. It was discovered in 1868 by Hofmann and was made by passing a mixture of air and methyl-alcohol (wood alcohol) vapor over heated platinum. It is practically all obtained by the oxidation of methyl alcohol. This is ac-

complished in "Formaldehyde Lamps," a platinum cone heated by electricity. It is a gas which is condensed to a colorless liquid at 21° C., and at a higher temperature is changed into paraformaldehyde, which is the commercial article sold for preserving purposes in 40 per cent solutions. A 50 per cent solution is made, but is unstable.

The germicidal power of formaldehyde was discovered in 1888 by Traillat, who patented a process for manufacturing it. The germicidal power is nearly equal to that of corrosive sublimate and is greater than that of carbolic acid. A 1 per cent aqueous solution kills all spores of pathogenic bacteria in one hour. It decolorizes organic matter, precipitating extracts and colors.

It is a strong irritant when inhaled and affects the mucous membrane of the mouth and throat and inflames the nasal passages; the gas also irritates the eyes and causes them to smart and water. It passes through the body when taken in food and the urine does not ferment; it will deodorize faeces or putrefactive products; it is an excellent disinfectant and may be heated over a lamp to generate gas for disinfecting rooms after cases of contagious and infectious diseases; it has been used to embalm bodies and gives firmness to the flesh.

It does not exert a very great germicidal power over molds and yeasts and for this reason was a failure as a substitute for salicylic acid as a preservative for tomato products, such as catsup, Chili-sauce, chutney, etc. It has entered into the composition of various "trade preservatives" and has thus been the cause of considerable trouble which manufacturers have had with food commissioners. The manufacturers have been purchasing various antiseptics under *trade names* designated by numbers, which are either simple or complex antiseptic chemicals, and are put up and sold under disguised names at four to ten times their actual value. Many of these contain formaldehyde, benzoic acid, boric acid and sometimes salicylic acid and oftentimes other well-known chemicals which have only very mild antiseptic power.

Formaldehyde is probably injurious when used to any very great extent in foods. Owing to its peculiar nature it hardens cellular and albuminous matter and should not be used as a preservative for meats, fish and many vegetables, but it seems to preserve milk 1 to 15,000 for a few days without any serious chemical changes.

The prevalence of this chemical in nature is remarkable, although the amount usually present in various plants and manufactured foods is perhaps small. It is present in many of the growing plants; it is a product of vital action of bacteria on vegetable matter; it is found frequently in fresh milk and nearly always in milk

which has stood exposed to the action of bacteria. Some vegetables, such as peas, beans, asparagus, sugar corn, etc., when allowed to stand exposed to the air before canning, will give the chemical reaction for formaldehyde.

Many vegetables and meats, when processed in steam retorts at 250° F., show the presence of formaldehyde by the official test. At this place I wish to state that any person who has made careful analyses for formaldehyde in nature will be able to judge whether the reaction is due to an added chemical or merely a natural formation. While there is no reliable official quantitative analysis, yet the analyst should be able to tell by the strength of the reaction whether formaldehyde has been purposely added as a preservative or whether it is there naturally or as a result of oxidation.

There are some substances which give reactions so closely resembling those of formaldehyde that the analyst must be extremely careful in forming conclusions. It is a lamentable fact that some of our agricultural chemists have fallen victims to both of the wrong conclusions cited. It has been suggested that perhaps the raw material purchased from other houses probably contained formaldehyde or some other antiseptic, and the food manufacturer, while innocent of adding this preservative himself, has unconsciously been guilty of statute violation; so we will give the official test for formaldehyde to enable him to make analyses of all raw material used to make up a given product.

CHEMICAL ANALYSIS FOR FORMALDEHYDE. (W. M. ALLEN.)

In case the suspected material is solid or semi-solid, macerate from 200 to 300 grams with 100 c. c. of water to obtain sufficient fluidity. Make this preparation distinctly acid with phosphoric acid and fill into flask of 500 to 800 c. c. capacity. A copper flask may be heated directly over flames but a glass flask is better heated in a linseed oil bath. Connect flask with glass condenser and distill off about 40 or 50 c. c.

If the suspected material is a liquid acidify as before and process as directed under benzoic acid to obtain a chloroform or ether extract, which is distilled in oil bath at 250 to 260 degrees F.

METHOD NO. I.

To about 5 c. c. of the distillate add two or three drops of a 1 per cent aqueous solution phenol; mix and carefully pour it on about the same amount of sulphuric acid in a test tube, holding tube so that the solutions will *not* mix. The presence of one part of formaldehyde in 100,000 parts is indicated by the formation of a crimson color at the place of union of the solutions. If formaldehyde be

present in greater quantity a white turbidity or a light-colored precipitation will be formed above the coloring.

If organic matter is distilled over the charring of it by the sulphuric acid may be mistaken for a trace of formaldehyde, but on allowing the test to stand for twelve hours the coloration due to the formaldehyde will become a whitish turbidity instead of the dark color which appears if due to the charring of organic matter.

Note.—Some other aldehydes will give the same result and it is not therefore conclusive.

METHOD NO. 2.

Add about 5 c. c. of the distillate obtained originally to an equal volume of pure milk in a casserole, also 10 c. c. of muriatic acid (C. P.) containing 1 c. c. of a 10 per cent solution of ferric chlorid solution to each 500 c. c. of acid. Heat to 80° or 90° directly over flame, giving casserole a rotary motion to break up the curd of the milk. A violet color may indicate formaldehyde.

METHOD NO. 3.

Dissolve 1 gram of phenylhydrazin hydrochlorid and 1½ grams acetate of sodium in 10 c. c. of water. To 1 c. c. of distillate obtained originally add 2 drops of reagent and 2 drops of sulphuric acid. A green color indicates the presence of formaldehyde.

BORACIC ACID OR BORIC ACID.

Boric acid is a preservative used for milk, meats and vegetables. Various preparations of boric acid or borates are sold under trade names as food preservatives. A mixture of boric acid and borax was sold under the name of "Rex Magnus;" another mixture of boric acid and glycerol is sold under the name of "Boroglycerid."

Boric acid is symbolized as H_3BO_3 and is obtained by the interaction of sulphuric acid (H_2SO_4), and borax ($Na_2B_4O_7$), also by the purification of native boric acid, found in combinations as a magnesium salt in sea water, mineral waters, such as Vichy, Wiesbaden and Aix-la-Chapel; also in mineral substances, as boro-calcite in the niter beds of Chili; also in natural borax or tincal in the dried-up lagoons in central Asia; in large quantities in Clear Lake, California. It is found in ulexite (sodium and calcium borate) and colemanite (calcium borate), also found in a large vein deposit, probably of volcanic origin in San Bernardino County, California, which yields about twenty-five million pounds annually.

Boric acid occurs as pearly scales soluble in water and alcohol, has a feeble acid reaction and possesses a bitter taste. It changes

to metaboric acid (HBO_2) when heated to 250° F. and may be changed by further heating to its anhydrid, B_2O_3.

As an antiseptic it has very little value and has, I think, been very much over-estimated, although it is used with fair success for preserving milk. When combined with other salts it seems to retard putrefaction in meats, sausages, butter and milk. It is never used in sufficient quantities to be germicidal, hence pathogenic bacteria, such as typhoid, anthrax, hog cholera, tuberculosis, etc., will remain alive, though dormant, in meats lightly cured and in butter made from infected cream.

Much of our exported meat, butter and other foodstuff is partially preserved with boric acid, and this seems to be necessary for countries which are not advanced in refrigerating methods. Our manufacturers use large quantities, therefore, in expert goods to prevent reclamations on account of spoilage. This is not so necessary in our country, because we have means of preserving such foods in refrigerators or cold storage.

As a preservative for catsup, Chili-sauce, chutney, jams, jellies, preserves, etc., boric acid has very little value. When Barff discovered "boro-glycerid" in 1886 it was hoped that it might be a valuable harmless antiseptic for tomato products, but the tests did not give satisfaction.

Boric acid is antiseptic in 1 part to 300.

OFFICIAL CHEMICAL TEST FOR BORIC ACID.—NO. 1, QUALITATIVE ANALYSIS.

Render decidedly alkaline with lime water about 25 grams of the sample evaporate to dryness on a water bath. Ignite the residue to destroy organic matter. Add about 15 c. c. of water and hydrochloric acid, drop by drop, to acid reaction. Then add about 1 c. c. of concentrated hydrochloric acid. Moisten a piece of delicate tumeric paper with the solution; if borax or boric acid is present the paper on drying will acquire a peculiar red color, which is changed by ammonia to a dark blue-green, but is restored by acid. This color is almost unmistakable, but it is best for one not familiar with it to conduct a test where boric acid is known to be present.

NO. 2.—QUALITATIVE ANALYSIS. (OFFICIAL.)

Add an equal drop of fresh saturated tumeric tincture and a drop of hydrochloric acid and heat for a few seconds.

If the suspected material be a liquid, evaporate with the tumeric and heat with a drop of diluted hydrochloric acid for a few seconds; then if borax or boric acid be present a pink or dark red color will appear, depending upon the quantity present. Cool and add a drop of ammonium hydroxid, when a dark blue-green color will appear.

MIQUEL'S TABLE OF ANTISEPTICS.

Miquel made tests of a large number of substances to ascertain their antiseptic value, many are powerful poisons for man as well as bacteria. There are quite a number whose action on the human organism are not positively known, and there are quite a number which have only very slight antiseptic power. The most common powerful antiseptic and disinfectant in general use for destroying bacteria on instrument, furniture, and various materials, which do not come in contact with food, is mercuric chlorid, or corrosive sublimate. This is used largely in our laboratories for destroying cultures of bacteria, or for disinfecting purposes. We give Miquel's table of antiseptics and their proportions which prove effective.

SUBSTANCES EMINENTLY ANTISEPTIC.

Mercuric iodid	1 part in		40,000
Silver iodid	1 "	"	33,000
Hydrogen peroxid (this is unstable)	1 "	"	20,000
Mercuric chlorid	1 "	"	14,300
Silver Nitrate	1 "	"	12,500

SUBSTANCES VERY STRONGLY ANTISEPTIC.

Osinic Acid	1 part in		6,666
Chromic Acid	1 "	"	5,000
Chlorine	1 "	"	4,000
Iodine	1 "	"	4,000
Chlorid of Gold	1 "	"	4,000
Bichlorid of Platinum	1 "	"	3,333
Hydrocyanic Acid (Prussic Acid)	1 "	"	2,500
Bromine	1 "	"	1,666
Cupric Chlorid	1 "	"	1,428
Thymol	1 "	"	1,340
Cupric sulphate	1 "	"	1,111
Salicylic Acid	1 "	"	1,000

SUBSTANCES STRONGLY ANTISEPTIC.

Benzoic Acid	1 part in		909
Potassium Bichromate	1 "	"	909
Potassium Cyanid	1 "	"	909
Aluminum Chlorid	1 "	"	714
Ammonia	1 "	"	714
Zinc Chlorid	1 "	"	526
Mineral Acids	1 "	"	500 to 333
Thymic Acid	1 "	"	500

Lead Chlorid	1	"	"	500
Nitrate of Cobalt	1	"	"	476
Sulphate of Nickel	1	"	"	400
Nitrate of Uranium	1	"	"	356
Carbolic Acid	1	"	"	333
Potassium permanganate	1	"	"	285
Lead Nitrate	1	"	"	277
Alum	1	"	"	222
Tannin	1	"	"	207

SUBSTANCES MODERATELY ANTISEPTIC.

Bromhydrate of Quinine	1	part	in	182
Arsenious Acid	1	"	"	166
Boracic Acid	1	"	"	143
Sulphate of Strychnia	1	"	"	143
Arsenite of Soda	1	"	"	111
Hydrate of Chloral	1	"	"	107
Salicylate of Sodium	1	"	"	100
Ferrous Sulphate	1	"	"	90
Caustic Soda	1	"	"	56

SUBSTANCES FEEBLY ANTISEPTIC.

Perchloride of Manganese	1	part	in	40
Calcium Chloride	1	"	"	25
Sodium Borate	1	"	"	14
Muriate of Morphia	1	"	"	13
Strontium Chloride	1	"	"	12
Lithium Chloride	1	"	"	11
Barium Chloride	1	"	"	10
Alcohol	1	"	"	10

SUBSTANCES VERY FEEBLY ANTISEPTIC.

Ammonium Chlorid	1	part	in	9
Potassium Arsenite	1	"	"	8
Potassium Iodid	1	"	"	7
Sodium Chlorid	1	"	"	6
Glycerine (sp. gr. 1.25.)	1	"	"	4
Ammonium Sulphate	1	"	"	4
Sodium Hyposulphite	1	"	"	3

A careful study of this table shows that the antiseptics usually employed in foodstuff are equally effective with some of the most powerful mineral poisons known. Miquel places hydrogen peroxid as third in the list of antiseptics eminently powerful. If this

preparation were stable it could be used almost ad libitum, because it is not poisonous, but it loses its properties rapidly and soon decomposes into water by giving up one atom of oxygen, thus H_2O_2—$O = H_2O$.

From our experience with this substance we are inclined to think that Miquel overestimated its value as an antiseptic. It does not prove effective as a food preservative. Nearly all my experiments have failed, even when the proportion used was 1-1,000. This may be due to its unstable nature, and cannot therefore be satisfactory.

CHAPTER X.

Artificial Sweeteners and Adulterants

Saccharin. Methods of Detection. Dulcin. Methods of Detection. Glucin. Sulphites. Methods of Detection. Artificial Colors. Starch, etc.

SACCHARIN.

Saccharin is the commercial name for Glusidum, or, according to the British, the drug name is given as Gluside; the chemical name is benzoyl sulphonimide, which gives some clue to its base origin. It is a sweet imide, from toluene, and is symbolized as $C_7H_5NSO_3$. It is obtained by first converting toluene into sulphamide, which by oxidation yields the imide. It forms a white powder which melts 392° F., with partial decomposition, evolving the odor of bitter almonds. It is soluble in water, from which it may be crystallized in alcohol, ether, glycerin and glucose. Saccharin may be detected in solutions containing sugar, by extracting with ether, then evaporating and fusing the residue, which will melt at about 392° F., and if fused with nitre and carbonate of sodium, will show sulphuric acid. The weight of $BaSO_4$ obtained in this way from 100 grams of sugar multiplied by 0.785 will give weight of saccharin extracted.

Saccharin occurs as a white powder composed of irregular crystals only slightly soluble in water, readily soluble in glycerine, alcohol and ether. The aqueous solution has a distinct acid reaction and forms salts.

The commercial saccharin contains para-sulphamine-benzoic acid, from which impurity it may be freed by recrystallization, acetone being used as the solvent. The difference in the melting point between saccharin and para-sulphamine-benzoic acid is also used for distinguishing them. The pure chemical saccharin melts at 286.5° F., while the other melts at 224.5° F.

Saccharin is very soluble in weak solution of ammonia, also in bicarbonate of sodium.

Saccharin has no properties of sugar except sweetness; it has no food value, and passes unchanged through the kidneys, and will prevent to some extent ammoniacal fermentation of the urine.

As an antiseptic it has very little value, and is not used in food products for preserving or preventing fermentation. Wherever it

is used, the object is to take the place of sugar. It is generally regarded by Pure Food Authorities as an adulterant, and this is one construction that can be put upon its employment in food products. That it is used in large quantities for sweetening glucose, syrups, preserves, jams, jellies and canned goods, such as corn and peas, cannot be denied. The consumers in all cases, no doubt, believe that their goods owe their sweetness to sugar, and are thus deceived and are deprived of the food which they believe they are purchasing, sugar being a food and saccharin having no food value. There are large quantities of syrups almost worthless as such, which are sweetened with saccharin and sold at fair prices, the consumers believing same to be the product of cane sugar.

There are many authors quoted on each side of the question, "Is saccharin injurious to the human organism?" and it is not within the province of this work to enter into the discussion, but we believe that the preponderance of evidence is unfavorable to its employment as a sweetener of food products. Among the authorities who write against it, might be quoted Dr. Wiley, Chief Chemist of the United States, Thomson, Sollman, Dr. Butler, Dr. C. H. Wood and Paul. These authorities claim that "Saccharin checks the action of ptyalin, pepsin, trypsin and other allied ferments," that "it increases the amount of chlorides excreted from the urin," that "it has no food value; it is an antiseptic; it prevents decay, and therefore retards digestion to that extent." It is believed by some that its long continued use may give rise to nephritis.

From the knowledge that we possess, viz., its employment as a substitute for cane sugar, and the possible injurious effects which it may have upon the human organism, it seems wise that every packer of foodstuff eliminate saccharin and make every effort to elevate the standard of their goods by using only cane sugar as a sweetener.

There are several tests which are used to determine the presence of saccharin, and the packers who use syrups in their formulas may determine its presence as follows:

If the sample to be tested is a solution or syrup, render it acid, if not already such, with phosphoric acid, and extract with ether. In case of canned vegetables and similar goods, finely divide the material by pulping or maceration in a mortar, dilute with water, and strain through muslin. Acidify the filtrate, and extract with ether. If an emulsion forms, use a centrifugal machine. Separate the extract, evaporate off the ether, and test the residue for saccharin as follows:

(1) Add to the residue, if it tastes sweet, a few cubic centimeters of hot water, or preferably a very dilute solution of sodium carbonate, in which saccharin is more soluble. An intensely sweet

taste is indicative of its presence. This test, if applied directly, will sometimes fail, especially in the case of beer, by reason of the extraction of ether of various bitter principles, such as hop resins, which by their strong, bitter taste mask the sweet taste of saccharin in the residue. Speath recommends that such bitter substances be removed before extraction, which is done by treatment of 500 c. c. of the beer with a few crystals of copper nitrate, or with a solution of copper sulphate. The flocculent precipitate formed need not be filtered off, but the liquid is preferably concentrated by evaporation to syrupy consistency, acidified with phosphoric acid, and extracted with three successive portions of a mixture of ether and petroleum ether. After extraction, separation, and evaporation of the solvent, dissolve the residue in weak sodium carbonate. As small a quantity as 0.001 per cent of saccharin can be detected in the final alkaline solution by its sweet taste.

(2) Bornstein's Test.—Heat the residue from the ether extraction of the acidified sample with resorcin and a few drops of sulphuric acid in a test tube till it begins to swell up. Remove from the flame, and, after cooling till the action quiets down, again heat, repeating the heating and cooling several times. Finally cool, dilute with water, and neutralize with sodium hydroxid. A red-green fluorescence indicates saccharin. Gantter states that it is useless to apply this test to beer, in view of the fact that ordinary hop resin gives the same fluorescence.

(3) Schmidt's Test.—The residue is heated in a porcelain dish with about a gram of sodium hydroxid for half an hour at a temperature of 250° C., either in an air-oven or in a linseed oil bath. This converts the saccharin if present into sodium salicylite. Dissolve the fused mass in water, acidify, and extract the solution with ether. Test the ether residue in the regular manner for salicylic acid with ferric chlorid. This test can obviously be applied only in the absence of salicylic acid, which should first be directly tested for.

DULCIN.

This white powder is composed of needle-like crystals, slightly soluble in cold water, ether and chloroform. Its symbol is $C_9H_{12}N_2O_2$. It is soluble in 800 parts of cold water, 50 parts of boiling water, and 25 parts of 95 per cent alcohol. It is soluble in acetic ether. Dulcin is about four hundred times as sweet as cane sugar.

When dulcin is combined with N/10 sodium hydroxid and subjected to distillation, a substance called phenetidin is formed which volatilizes and passes into the distillate. This, when heated with glacial acetic acid, forms phenacetin. Phenacetin is detected by first boiling with hydrochloric acid, diluting with water, cooling the filtrate, and adding a few drops of chromic acid solution. A deep red color is formed.

DETECTION OF DULCIN.

(1) Bellier's Method.—A portion of the sample to be tested is made alkaline and extracted with acetic ether. In the case of certain products it is best to subject them to varied preliminary treatment, depending on the case in hand. With such products as thin fruit syrups, simply make alkaline and shake out with acetic ether. In the case of thick fruit syrups, confectionery and preserves, dilute with water, add an excess of basic lead acetate, remove the lead by precipitation with sodium sulphate, filter and make the filtrate alkaline.

Having thus obtained a clarified solution, use from 50 to 200 c. c. of neutral acetic ether to say 500 c. c. of the alkaline solution, and shake in a separatory funnel. Separate the extract, filter, and evaporate to dryness. If the dulcin exceeds 0.04 gram per liter, crystals will be apparent in the residue. If fats and resins are present in the residue, make repeated extractions with hot water, and evaporate to dryness. The purified residue is finally brought to dryness in a porcelain dish, and treated with 1 or 2 c. c. of sulphuric acid and a few drops of a solution of formaldehyde. Let it stand for fifteen minutes, and afterwards dilute with 5 c. c. of water. A turbidity or precipitate indicates dulcin.

(2) Jorissen's Test.—The residue from the acetic ether extract of an alkaline solution of the sample is treated with 2 or 3 c. c. of boiling water in a test-tube, and a few drops of mercuric nitrate are added. Heat the tube and its contents for five minutes in a boiling water bath, withdraw, and disregarding any precipitate, add a small quantity of lead peroxide. On the subsidence of the precipitate, which quickly occurs, a fine violet color appearance forms for a short time in the clear upper layer in presence of 0.001 gram of dulcin.

(3) Morpurgo's Method.—To the acetic ether residue, evaporated to dryness in a porcelain dish, add a few drops of phenol and concentrated sulphuric acid, and heat a few minutes on the water-bath. After cooling, transfer to a test-tube, and with the least possible mixing pour ammonia or sodium hydroxid over the surface. A blue zone at the plane of contract between the two layers indicates dulcin.

GLUCIN.

This is a light-brown powder soluble in water, but not in ether and chloroform. It is three hundred times sweeter than cane sugar. Its symbol is $C_{19}H_{16}N_4$.

DETECTION OF GLUCIN.

Dissolve in dilute hydrochloric acid and cool, then add a few drops of sodium nitrite solution, followed by a few drops of an alkaline solution or beta-napthol. A red color is produced. If resorcin or salicylic acid is used instead of beta-napthol, the color will be yellow.

SULPHITES.

Sulphurous acid, H_2SO_3, in the form of SO_2, sulphur dioxid, is combined with soda and used as a preservative and as a bleaching agent. The sulphites are bisulphite of soda and hyposulphite of soda, the former being used largely in preserving meats and as a bleaching agent for corn and asparagus, also all kinds of dried fruits. Sulphur dioxide is widely distributed in nature and is present in minute quantities in various fruits and vegetable, and qualitative analytical tests for its presence must show more than a trace in order to establish the fact that it has been employed as a preserving or bleaching agent. That this substance is harmful to the human organism is doubtful. The quantity which may be used in food products is necessarily small since it imparts a sulphurous taste if used in excess. That sulphites are necessary in any branch of the food industry is doubtful. Corn bleached with them becomes tasteless and loses a great deal of its flavor; asparagus is better with a natural color. Used as a bleach for evaporated fruits it may appear to be necessary, but if the public be educated to accept the natural fruit, its necessity will disappear. As a preventive of mold sulphurous acid is effective and may be recommended for cleansing the packages and utensils employed in the food industry.

For the detection of sulphites we give the official test, so that packers may analyze their own raw materials procured from outside sources. A microscopical inspection will show the presence of the crystals on raisins, currants, citron, etc., used in mince-meat and other preparations. If the sulphites are present on the raw material, of course the finished product will give the chemical reactions and may be condemned by the food commissioners.

METHOD NO. I—BY DISTILLATION.

Place 50 grams of the material in a distilling flask, add about 5 c. c. of a saturated solution of glacial phosphoric acid, add enough distilled water to make up 100 c. c., and distil in a current of carbon dioxid until 50 c. c. have passed over. Take a few c. c. of the distillate, add a slight excess of iodin solution, boil to expel excess of iodin, then acidify with hydrochloric acid and add barium chlorid solution. This test is very delicate and is easily applied.

METHOD NO. 2—BY REDUCTION.

To about 25 grams of the sample placed in a 200 c. c. Erlenmeyer flask, add some pure zinc and several cubic centimeters of hydrochloric acid. In the presence of sulphites, hydrogen sulphid will be generated and may be detected by lead paper. Traces of metallic sulphids are occasionally present in vegetables, and by the above test will indicate sulphites. Hence positive results obtained by this method should be verified by the distillation method.

It is always advisable to make the quantitative determination of sulphites, owing to the danger that the test may be due to traces of sulphids. A trace is not to be considered sufficient evidence that a sulphite has been used either as a bleaching agent or as a preservative.

The chemicals described are those principally employed as preservatives of food. There are many more powerful in their germicidal power than these, but are either known poisons or are unstable and consequently cannot be employed as food preservatives. There are, however, various materials used in preparing raw materials and even finished products, which are preservatives in a slight degree, although not always employed for that purpose. Common salt is a mild preservative and if used in the form of brine restricts fermentation, allowing only certain microorganisms to flourish, which generally belong to the lactic acid group, while the bacteria which elaborate foul products and gases are completely checked, first by the salt, and then by the lactic acid formed.

Sugar, when used in sufficient quantities, is a preservative, because it rapidly takes up the fluids to form syrups, and all bacteria are deprived of the moisture so necessary for reproduction or vegetation. Small quantities of sugar have no antiseptic value, since the carbon is rapidly utilized to supply that element so necessary for the development of cell protoplasm, of bacteria. Thus small quantities of sugar are favorable to the growth of yeasts and molds, also various species belonging to the schizomycetes or fission fungi. Sugar, when split up by fermentation set up by yeasts and molds, is converted into alcohol, glycerin, carbonic acid, succinic acid, and other fatty acids, or it may be attacked by lactic acid bacteria and be split up into lactic acid without the evolution of gas. Sugar, when used as a heavy syrup, is antiseptic to a considerable degree, since most bacteria cannot obtain sufficient fluid to form new protoplasm.

Acetic acid is employed as a preservative in the form of vinegar, but some vinegars containing large quantities of organic matter, do not possess much antiseptic power. Some vinegars are themselves easily attacked by bacteria and their acetic acid is changed to carbonic acid and water. The best pickling vinegar is white wine, obtained by distillation; cider, malt and fruit vinegars are very

susceptible to changes if exposed to warm temperatures. The 5 per cent white wine vinegar has antiseptic power and is largely used in all pickled goods.

ARTIFICIAL COLORS.

There are several reasons given by some manufacturers for the employment of artificial colors to brighten their goods; some claim that the uncolored product does not look appetizing, therefore it should be colored just enough to please the eye. In a sense we all eat with our eyes, and it is a question whether food has the same value if it does not appeal to the eye. Several experiments have been tried upon animals by feeding them blindfold and the results gave evidence that the food did not accomplish its full value, for the animals grew weak and emaciated. Some claim that colors should be used to cover up certain defects which cannot be avoided in present methods of manufacture. This claim is based on the discoloration of raw material, which is stored away in barrels and other wooden packages. It is claimed that during the busy season the fresh products from the farms are delivered to the packers much faster than they can be worked into finished goods, and this necessitates the storing of partially prepared material in barrels and casks, until such time as may be more convenient for finishing. The tannic acid from the wood discolors this material very much, and the packers claim that it is necessary to restore its natural appearance by adding certain coal tar dyes which give the finished product the appearance of freshly prepared stock.

We all know that vegetables and some fruits lose some of their natural color during the heating which is necessary to properly sterilize them; in some cases the color is changed slightly, though not faded; corn is an example of this. Peas, string beans, asparagus, catsup, Chili-sauce, tomato chutney, fruit juices and many other raw materials lose a certain amount of their natural color in the sterilizing process and in the cooking or evaporating methods, but if these materials are prepared properly they will still retain enough of their original color to appeal to the palate, and do not need the addition of coal tar dyes or other colors to replace the small amount of natural color lost during the preparation.

Packers, who contract for more raw material than they are able to make up into finished goods, should not try to imitate first-class goods with the surplus material which they are forced to accept, unless they have some good method for keeping it. No packer should contract for more raw material than he is able to handle properly, and if he does receive more and is forced to save it by partial cooking and storing in wooden packages, he should expect

to sell same for its true value, and not attempt to bring up the standard by artificial colors. Now to make this clear, we will take for example one packer who contracts just what tomatoes he thinks he is able to make up into catsup, Chili-sauce, etc., during the season and he employs all the help that is necessary to finish the goods and bottle same without having to store away any pulp, in other words, he invests at once in everything necessary to finish and take care of his daily receipts of raw material, and has that investment tied up for six or eight month; his goods are not artificially colored nor do they need to be, and the quality will be the very best, but he goes into the market and finds goods just as bright, or perhaps brighter than his, which have been prepared from barreled stock and artificially colored. The consumer, who does not know the circumstances, perhaps purchased the brighter goods, thinking that the quality will compare favorably with the color. Now this is unfair to the other man, and he is discouraged in his efforts to manufacture pure goods simply because he has no protection. In order to get the very best goods it is necessary to protect the packers against every imitation; then the inferior goods will show by their color that they have not been made from strictly fresh stock. The question then arises, "What shall be done with our surplus material which may accumulate, despite our best efforts to take care of it as fast as it comes in?" There should be arrangements made to put on extra help for such contingencies, or if this is impossible, the surplus stock should be canned in large size packages, such as five or eight gallon tin cans, and then sterilized in boiling water; this applies nicely to tomato pulp and if it be put away in this manner, it will open up nearly as fresh and bright as when first canned. Fruits contracted for preserving should be made up into finished goods at once, and if properly handled, will need no color to make the finished goods look well. There is always enough natural color in fruits to give a good appearance to jams, jellies and preserves, and it is pretty certain that such products when colored, are either adulterated, or they are prepared from stock which has been stored away in packages which have discolored the contents. Now if all this be true, is it not fair that the packer who prepares for the manufacture of his good directly from fresh stock and who employs the necessary labor and invests his money at once should have protection? If anyone stops to think of the result of measures to protect first-class goods, he can readily see that there will be an incentive to pack only first-class goods. *One unit of inferior goods is much more difficult to force into consumption than ten units of first-class goods.* If every packer will elevate his standard to the very best, there will be ten times the amount consumed. The great mass of the people use only sparingly of manufactured canned goods, preserves, jellies

and food products in general, but let them feel confident that they are getting just as good, or perhaps better goods, than they prepare at home, and the demand for all kinds of food products will be wonderfully increased. For a time perhaps this will be difficult and expensive for some packers who are not prepared to pack all of their daily receipts of raw material into finished goods at once, but it will probably have to be done to comply with Pure Food Laws. To do this perhaps it may be necessary to contract for less produce, or it will be wise in any event to prepare for storing raw material partially finished in large size tin cans which may be sterilized.

Some of the goods imported into the United States are very highly colored, so much so that they appear unnatural, and the Department of Agriculture has taken steps to stop the sale of such goods. Much of the blame for coloring our own goods is due to the imported goods; our manufacturers have been trying to imitate them because there seemed to be a popular belief that such goods were better than our own.

This impression, no doubt, was made by the beautiful colors worked in by the French artists. There are no artificial colors so delicate and beautiful as those painted by nature and the American people are learning how to detect the difference. As a matter of fact, let me say that there are very few brands of imported goods that can compare in any way with those of some of our best home manufacturers. Our goods are not colored and perhaps not as uniform in sizes as the imported, but for flavor and natural color they far surpass those sold as fancy imported goods. This is true of the products of many other industries and I would not be surprised that our foreign friends should soon begin to take lessons from our manufacturers, indeed they are doing so to some extent now.

The hostile attitude of our Food Commissioners against the employment of colors in foodstuff has raised quite a storm among some manufacturers, but if we reason the matter, we must admit that an anti-color crusade is bound to correct many existing evils and will in the end be the means of elevating the standard of manufactured foods.

The profits on first-class goods are proportionately greater than on cheap goods, and as much money may be realized on a smaller pack of first-class goods as on a larger pack of cheap goods, and the general satisfaction which such goods give will make business a pleasure. The packer who is conscious of the fact that his goods are pure and wholesome, prepared from selected raw material in the best possible manner knows that each package will make for him a friend of every consumer, and that means increased demand for his goods and a reputation for quality.

Some of the raw materials used by our packers may be colored and adulterated and we will give the outlines for making analyses to determine the presence of adulterants. For the complete study of dyes and colors our readers are referred to such works as "Schultz and Juliuson Organic Coloring" and "Allen's Commercial Organic Analysis," but in a work of this nature we can only mention some of the most important methods of color analyses.

ARTIFICIAL COLORS.

ANALYSES BY OFFICIAL METHODS.

The separation and identification of artificial colors in foods and raw material, which is used to make up a finished food product, is rather difficult in some cases, because the quantity employed is usually small, and becomes more or less mixed with the natural colors. In order to identify artificial colors, they must be separated in a pure state and then tested.

Coal tar dyes are identified by the double dyeing method, and there need be no fear of mistaking them, if due care is exercised. The extraction of colors simply by the amyl alcohol method, does not signify that those colors are of coal tar origin, since many of the natural fruit colors may be thus extracted, and it is possible to dye wool permanently with some of them.

Coal tar dyes are poisonous, from the fact that they become contaminated with such metals as zinc, tin, lead and arsenic, the last being present in the sulphuric acid, which is usually employed in the manufacture of the dyes. Some of these dyes contain metals, such as malachite green, which is a double chlorid of zinc, in combination with the organic group. Many so-called vegetable colors are sold as lakes of tin or alum. Some colors contain picric acid, and naphahol yellow, and these are known poisons.

Combinations of dyes are sometimes used, and are difficult to determine; they are detected by a system of fractional dyeing, the fabric taking the different dyes at different rates of time.

DETECTION OF COAL TAR DYES IN TOMATOES AND TOMATO PRODUCTS.

Extract the color from dried pulp with ordinary alcohol and acidify with hydrochloric acid and filter. Eosin, which is most commonly used, gives a fluorescent filtrate. Dilute the filtrate with water, extract with amyl alcohol, and dye. Cochineal, if present in the form of a lake, will require strong hydrochloric acid to decompose it. Separate the amyl alcohol, and wash until neutral. Then divide into two portions, to the first add drop by drop, a very dilute solution of uranium acetate, and shake thoroughly each time.

The presence of cochineal is indicated by a characteristic emerald green color. To the second portion, add a drop or so of ammonia, and the presence of cochineal is indicated by a violet color.

DETECTION OF ARTIFICIAL COLORS IN PEAS, BEANS, GHERKINS, ASPARAGUS, ETC.

Copper salts are often used to give color to these vegetables, and sometimes zinc is also employed. Reduce 15 to 20 grams of the suspected sample to an ash, transfer the ash to a beaker, and treat with nitric acid; filter, make alkaline with ammonia, and if a precipitate forms, filter again. A blue color indicates the presence of copper. To confirm this test, acidify the filtrate with acetic acid, add potassium ferro-cyanide. Red coloration or precipitate indicates the presence of copper, and verifies the first test.

DETECTION OF TUMERIC IN VARIOUS PRODUCTS.

Extract the color from suspected sample with alcohol. Dip a piece of filter paper into this tincture and dry at 212° F., after which moisten with weak solution of boric acid, to which a few drops of hydrochloric acid has previously been added. Dry again, and a cherry red color will indicate the presence of tumeric. This color is characteristic and may be learned by conducting a test on a known tumeric colored sample.

DETECTION OF COAL TAR DYES IN JELLIES, JAMS, PRESERVES, ETC. METHOD NO. I. (SOSTEGNI AND CARPENTIERI.)

From 10 to 20 grains of the sample are dissolved in 100 c. c. of water, filtered if necessary, acidified with 2 to 4 c. c. of 10 per cent solution of hydrochloric acid, and a piece of woolen cloth, which has been washed in a very dilute solution of boiling potassium hydroxid and then washed in water, is immersed in it and boiled for five to ten minutes. The cloth is removed, thoroughly washed in water, and boiled with a very dilute hydrochloric acid solution. Then after washing out the acid, the color is dissolved in a solution of ammonia hydroxid (1 to 50). With some of the dyes the solution takes place quite readily, while with others it is necessary to boil some time. The wool is taken out, a slight excess of hydrochloric acid is added to the solution, another piece of wool is immersed and again boiled. With natural vegetable coloring matter this second dyeing gives practically no color, so there is no danger of mistaking vegetable colors for coal tar colors. *It is absolutely necessary that the second dyeing should be done,* as some coal tar dyes will produce a dirty orange in the first acid bath, which might easily be mistaken for a vegetable color, but on solution in

alkaline bath, the second acid bath will produce a bright pink color, indicating that the dye was of coal tar origin.

METHOD NO. 2. (ARATA.)

This method has particular value for the detection of coal tar colors in fruit products.

From 20 to 30 grams of the sample dissolved in 100 c. c. of water, are boiled for ten minutes with 10 c. c. of a 10 per cent solution of potassium bi-sulphate, and a piece of white wool or woolen cloth, which has been previously boiled in a very dilute solution of sodium hydroxid and thoroughly washed in water is boiled in the solution. After removal from the solution, the wool is washed in boiling water, and dried between sheets of filter paper. If the coloring matter is a natural fruit color, the wool will either be uncolored, or will take on a faint pink, or brown, which is changed to green or yellow by ammonia, and not restored by washing.

In addition to this, it is advisable in all cases to dissolve out the coloring matter with ammonia, as in the first method, and dye again.

An advantage in the second dyeing is, that if a large piece of woolen cloth be used in the first dyeing, and a small piece in the second dyeing, small amounts of coloring matter may be brought out much more decidedly in the second dyeing, where practically all of the vegetable coloring matter has been excluded. For the identification of the various coal tar dyes, the reader is advised to consult special works on dyeing.

DETECTION OF COAL TAR DYES BY EXTRACTION WITH SOLVENTS.

METHOD NO. 3. (METHOD USED IN PARIS MUNICIPAL LABORATORY.)

The acid colors (sulphu-fuchsin, azo derivatives and phythaleins)., are not precipitated by tannin and are insoluble or only slightly soluble in acetic acid or amyl alcohol.

The basic colors (fuschsin, safranin, etc.) are precipitated by tannin, and are readily soluble in acetic ether and amyl alcohol.

No. 1. To 50 c. c. of suspected fruit liquid, add ammonium hydroxid in slight excess; then add 15 c. c. of amyl alcohol, shake and allow to stand.

(a) If the alcohol be colored red or violet, decant, wash, filter, evaporate to dryness in presence of a piece of wool, and test the dyed wool with sulphuric acid.

(b) If the alcohol be not colored, separate and add acetic acid. If the alcohol becomes colored the presence of basic aniline color is indicated.

(c) If the amyl alcohol be uncolored, both before and after the addition of acetic acid, no basic coal tar color is present.

No. 2. Add an excess of calcined magnesia, and then a 20 per cent solution of mercuric acetate, and bring to a boil. A coloration before or after addition of acetic acid indicates the presence of coal tar dyes, particularly acid dyes.

No. 3. Extract the solution with acetic ether made alkaline by barium hydroxid. This dissolves basic colors.

In any case the colors must be fixed on wool, for many of the fruit colors are extracted, and will give reactions with sulphuric acid, which might possibly be mistaken for coal tar dyes.

The double dyeing method will indicate clearly the difference between the natural vegetable or fruit colors and those of coal tar origin.

DETECTION OF CARAMEL.

Syrups, vinegars, sauces, vanilla extract and various other products are colored with caramel, in many cases to give the impression that the color is due to valued properties.

Ten c. c. of the solution to be tested are put into a high, narrow glass with perpendicular sides, as, for example, a small bottle; add from 30 to 50 c. c. of paraldehyde, depending on the intensity of the coloring, and enough absolute alcohol to cause complete mixing of the solutions. In the presence of caramel, a brownish yellow to dark brown precipitate will collect in the bottom of the glass. Decant the liquor, wash once with absolute alcohol, dissolve in small amount of hot water and filter. The color of this will give some idea of the quantity present.

Concentration by evaporation on steam bath is not allowable, since caramel will be formed. Any concentration must be done over sulphuric acid or at diminished pressure.

In order to further identify the color, it is poured into a freshly prepared solution of phenylhydrazin (2 parts phenylhydrazin-hydrochlorid, 3 parts sodium acetate and 20 parts water). The presence of a considerable quantity of caramel gives a dark-brown precipitate in the cold which is hastened by slightly heating. Small amounts require a longer time for precipitation.

DETECTION OF STARCH.

The packer often purchases various raw materials which he uses in the manufacture of specialties. Sometimes a good body is given to certain substances by the addition of starch. This, of course, deceives the packer, and his finished product is liable to be condemned because he did not know of the existence of starch in his goods. The writer has received (and tested considerable) cream which has been thickened in this manner.

Mustards are often thickened with wheat flour, and jellies are thus adulterated. There may be certain amounts of starch naturally in some of these substances, but a quantitative test will enable the packer to judge of this by analyzing a sample of known purity. Some unripe fruits will show the presence of considerable starch, but later this is converted into sugar during the ripening. For this reason good judgment is necessary in making tests for starch in products made from certain fruits. If the suspected sample has much color, this may be destroyed with sulphuric acid and permanganate of potassium. First heat the sample to nearly 212° F. and add a small quantity of sulphuric acid followed by permanganate of potassium until the color is destroyed. A few drops of tincture of iodin will give a blue color indicating the presence of starch.

There are various other raw materials used in special food formulas which are frequently adulterated, and it is necessary that the packers should know how to determine the presence of adulterants.

Our food manufacturers desire a better standard than ever before, and it is only a question of time when the employment of artificial colors and adulterants of all kinds will be looked upon with disfavor. Our efforts to bring out these points clearly may seem a little too far advanced, but undoubtedly there has been too much of this done, and, we believe, unnecessarily. Let us have our ideal strictly pure, wholesome, unadulterated food products, and earnestly strive to establish that standard.

Fruits and vegetables, jellies, preserves and other products whose color is easily affected, may now be put up in tin cans, coated or enameled on the inside, with a substance which is impervious to acids and is baked on the tin in such a manner, that a heavy sterilizing process does not remove it.

This inside coating is done by two firms, The Sanitary Can Company, of Fairport, N. Y., and The National Canning & Manufacturing Company, of Baltimore, Md. By using these cans, the problem of how to preserve the natural color of food products is solved.

CHAPTER XI.

The Canning Industry

A Short History. Location and Equipment of a Canning Factory. What to Can. Selection of Raw Material.

A SHORT HISTORY, PAST, PRESENT AND FUTURE.

In the year 1810 N. Appert, a Frenchman, published his work on canning. He had received a prize of 12,000 francs from the French government the year previous. About the same time Peter Durrand obtained a patent in England for a process of preserving fruit, meats and vegetables in tin cans are the patents granted to a Frenchman by name of Pierre Antoine Angilbert, in 1823. In America, the canning industry was started in Maine, by Isaac Winslow, in 1839, and about the same time Edward Wright began to pack oysters in Baltimore.

Winslow began packing corn, which was very fine in quality, and he succeeded in preserving a great deal by simply processing the cans for several hours in boiling water. His first experiments were made with corn on the cob, but he soon discovered that the sweetness of the kernels was absorbed by the cob, so he gave up the idea and cut the corn by means of a curved and gauged knife.

There are some records which seem to indicate that William Underwood began to pack certain foods in hermetically sealed packages, in Boston, Mass., about the same time, or prior to the establishment of corn packing in Maine. The records available prove that William Underwood did pack preserves and table condiments in glass, as early as 1828, and in 1836 he was packing tomatoes in glass, but there are probably no earlier records of goods having been packed in tin cans, than those of Isaac Winslow.

In 1860, the canning of corn, tomatoes, and fruits, was started by my father, Thomas Duckwall, near Cincinnati, Ohio. There is no record of any canning in the Middle States prior to that time, and thus Thomas Duckwall is recognized as the pioneer of the industry in that section.

Tin cans were difficult to make and owing to the crude apparatus for cutting tops and bottoms, the process was slow. A weight was pulled up to the ceiling and allowed to drop upon a sheet of tin; a die was cast on the under side of the weight, and the opposite die was cast in a piece of metal below, so that the forming of tops and

bottoms depended on the weight being properly guided. To make this operation as accurate as possible, the weight had two upright grooved guides, the same as those used to guide the weight in pile driving. My father made his first cans in this manner.

Canning of fruits and vegetables began in California about 1861-2, and to Francis Cutting belongs the honor of having been the first man to make and fill tin cans, although to his foreman, Alexander Young, belongs the credit of actually producing the results, from his practical knowledge. California proved to be a splendid country for growing all kinds of fruits and vegetables, so that canning factories sprang up all over the state, and the present output is enormous. The quality of goods manufactured is high, and the canning business occupies a very prominent place in the list of California industries.

The growth of the canning industry was rapid, after having been thus established in various parts of the country. New methods for making cans, improved machinery and skilled help quickly developed, and the increasing demand for the goods, gave the necessary impetus. In the eighties the growth of the industry was phenomenal, and new canneries sprang up like mushrooms all over the country, and the unskilled vied with the older-established packers, in the quantities of canned goods they could put up. The result was that a great deal of cheap, unwholesome goods soon flooded the market, and people became disgusted to the extent that they began putting up a large per cent of preserved and canned goods at home.

There were several causes for this reaction; the machinery men and promoters pushed their plans too fast, and unskilled men took charge of the canneries, and were soon packing all kinds of fruits and vegetable, and much inferior stuff resulted from inexperience. The wholesale grocers took a hand and began to squeeze the price down, at the same time requiring private labels which did not give the packer's name. Under such labels some very poor goods were manufactured, simply because the price was too low, and the fictitious label relieved the packer of the responsibility for the quality.

The result of low prices and loss of trade drove many canners out of the business, and the old established packers began with a determination *to make "quality" their aim.*

They began to give close attention to the selection of raw produce, forcing the farmers to furnish prime material, and to-day this is one of the most important factors in the production of first-class goods. Experienced men are in demand to supervise the work, and these are men who have not only a practical but also a scientific knowledge of canning and preserving. There is now a

much better class of canned food products offered to the trade than ever before, but for some time there have been flourishing numerous concerns who have been packing a very poor quality of goods, especially in the line of specialities. Some of these goods are so skillfully colored and preserved with antiseptics as to deceive the unsuspecting consumer. Thus we have the two extremes in manufactured food products, one representing the very best, purest and most wholesome, that modern knowledge and skill are able to produce; the other representing imitations of the better goods, and these are highly colored, adulterated and preserved by means of chemicals.

There has never been a time when strictly first-class food products were manufactured in excess of their demand by the trade. On the other hand the market has been repeatedly overloaded and injured by the cheap goods we have mentioned. The people, and particularly the masses, as a rule purchase the cheap goods, and the quality is often so poor as to disgust them, so the whole industry suffers much when the reaction comes. The masses are good advertisers of poor and also good food products and their condemnation or approval has power.

If the people of this country had implicit confidence in all manufactured food products, and all were strictly first-class, the maximum capacity of all the factories would be insufficient to supply the demand. If you take the yearly output of these factories, and figure out a pro rata for each person the result will convince you that there are wonderful possibilities for the food industry. This felicitous state of affairs can never be realized until all manufacturers enjoy the fullest confidence of the masses.

Having briefly outlined the history of canning and preserving from its beginning and having pried into the future, we may take a broad view of the present crusade against impure and low grade foods; it cannot be doubted that much good will result for the manufacturers. To be sure, if the laws are made excessively strict, some packers may be compelled to turn their attention to other lines of business, but those who comply with the conditions set forth in a strict national law will enjoy an unparalleled future demand for their goods, because home canning and preserving will decrease in proportion to the decrease of impure, unwholesome and adulterated goods.

How may this be done? Several ideas suggest themselves: First, there must be protection by a national law; second, strict compliance with the provisions of that law; third, a resolution to pack only the finest quality in cans or glass. To do this, it may be necessary to cut down the quantity of goods manufactured, in order that actual capacity may not be exceeded. The quality of

such goods will no doubt bring as large, perhaps larger, returns than the goods of poorer quality. It may be necessary to employ more skillful men; it may be necessary to lay aside a machine which crushes the fruits or vegetables according to present methods; it may be necessary to add improved machinery in one place and to employ hand work in another; it may be necessary to limit the selling territory; but whatever it may require, the end will justify the means, profits will be larger, and, while capital may be limited, a good reputation and a growing trade will generally be an inviting field for outside capital, if needed.

LOCATION AND EQUIPMENT OF A CANNING FACTORY.

The location of a canning factory is important, and the success of the business may depend entirely upon that, if everything else is all right. It is not enough to depend upon the ability to secure plenty of fruits, vegetables, etc. There must also be available help, and this is often a difficult problem in small places. Glowing accounts of fine crops and offers of free ground have led many to establish canning factories in various places, and the after failures were due to the difficulties experienced in securing sufficient and suitable help to take care of the stock delivered by the farmers. Improved labor-saving machinery has done much to remove this difficulty, but it is often not an easy matter to procure suitable persons to operate the machinery. Then again, certain machinery may save labor yet may so crush and bruise the stock that only a poor quality of goods may result. Even good machinery may be so poorly operated as to ruin the quality of the stock. Goods of fine quality cannot be produced in a factory where the stock is filled into the cans in a careless manner. Another important factor in selecting a location is the shipping facility. A canning factory should be near one railroad, and near two, if possible. During the season it is often necessary to receive supplies promptly, and the shipping of finished goods should be done with as little hauling by wagons as possible. Where two railroads are available the rates are generally lower and the service much better than where one line has complete control. As a rule, it is better to locate the canning factory as near as possible to the larger cities and towns; the difficulties experienced in obtaining farm products may be greater, but these are more than offset by the advantages in securing good help and shipping accommodations. Promoters of canning factories have been to blame in many cases for the establishment of these enterprises in so many unfavorable places; the many idle factories we see on country cross-roads are witnesses to the truth of our statement, and the proper location of the canning factory must ever be a matter of prime importance.

EQUIPMENT OF A CANNING FACTORY.

The proper equipment of the factory is a most important consideration. A poor equipment or one that is out of date not only increases the cost of packing, but also is a hindrance in the production of best quality. The building itself should be adapted to the particular line of goods manufactured and the arrangements for receiving raw material and the shipping should be made in such a manner as to avoid any back steps. Whenever goods have to be taken over the same space two or more times the cost of production is materially increased. This is usually the result of imperfections in building and should be overcome by making such additions as may be necessary to facilitate the work. It is no uncommon sight to see goods trucked up and down elevators two or three times before shipment. A proper storage place should be laid off on the same floor from which the goods are to be shipped. It costs considerable to load and unload trucks and there is always considerable time lost in waiting for elevators.

Every canning factory should have good boiler capacity, so that a nearly uniform steam pressure may be maintained without overfiring the boilers. The quality of a large per cent of food products depends in part upon uniformity of steam pressure. A pressure of about 90 pounds gives splendid results generally. The proper circulation of steam in the sterilizing retorts is important and cannot be as uniform when the boiler pressure falls to 40 to 60 pounds. There is always more or less condensation of steam at the optimum pressure, even when the pipes are covered with asbestos; but there is decidedly too much condensation at the low pressures mentioned.

Nearly all of the old packers have had considerable experience along this line, and some very severe cases of spoilage have been traced directly to improper steam circulation, due to low steam pressure in the boiler. For scalding tomatoes there should be sufficient steam pressure to make water boil vigorously. This will loosen the skin of the tomato and will not cook the fruit. After the proper scalding of tomatoes, the thin skin will peel off easily, and the tomato will be cool inside and will be firmer, and will hold its juice much better than when partially cooked during the scalding. Some factories have considerable waste in the peeling; the fruit had been partly cooked and a layer of the tomato would come off with the peel; this is due to poor steam.

For blanching purposes there should always be good steam pressure, and for all evaporating work the pressure should be high. The making of tomato catsup, Chili-sauce, etc., requires sufficient steam pressure to insure perfect circulation in the jacket of the ket-

tle; this will insure rapid evaporation and ebullition sufficiently strong to avoid scorching or sticking.

All goods which are to be cooked with live steam should have high pressure to avoid as much as possible the water of concondensation so copious in steam of low pressure. The water of condensation has a peculiar flat taste so often observed in distilled water, and this flavor is imparted to some goods, which greatly injures their quality.

To get the best results with steam, it is quite essential that all cooking should be done as near to the boiler room as possible. The nearer the cooking is to the boiler the less will be the water of condensation, and the better will be the circulation. If coal be used as fuel for boiler, the automatic feed is to be commended as a clean and labor-saving apparatus; it cannot always be used to advantage, however, because it requires a coal storage, one floor above the boiler room.

For power and lighting, the electric system is better and more economical than belting. The power may be carried to all parts of the building through small, well insulated wires, which may operate motors at the points most advantageous, and when not in use may be shut off, thus saving considerable energy. This system is especially attractive where the business is large and conducted on several floors or in different buildings.

The various products which are to be canned, preserved or bottled, generally require machinery specially constructed to do the work, and when it is known just what kinds of goods are to be prepared, it is a matter of judgment to determine what machines are the best for the purpose. There are machinery manufacturers who have special lines for the canning of all regular products, such as corn, peas and tomatoes, and the catalogues describe them fully. Some modern machinery, however, is built more for speed than quality, and it is impossible to produce strictly first-class goods. Special care must be taken to adopt no filling machine which in any way crushes the product to be canned. Some of the tomato fillers on the market crush and mash the fruit so badly that the contents afterwards appear more like slop than standard. It would be difficult to do all of this work by hand, nor is it necessary, because the consumer does not look for canned tomatoes firm and whole, unless he calls for such goods, which are classed as "fancy." It has been decided that the best tomato-fillers do not crush the fruit as much as the ordinary careless hand-filler. All fancy goods are filled by hand, however, and only uniform, selected tomatoes are used.

There are two systems for hermetically sealing tin cans. One used in European countries, and also in this country, seals the can

by crimping the top on to the flanged body, the sealing being made secure by a patented cement resembling rubber. This method has many advantages over the ordinary method of soldering; fruits and vegetables may be filled into the cans whole; there is no danger of getting any soldering solution inside the cans; there is absolutely no danger from lead poisoning, which is perhaps wrongfully blamed on the soldering system, although cans which are sealed with solder do not find favor in several foreign markets for this alleged reason.

With due care I believe that the wonderfully improved automatic capping machines, such as are used all over this country, will seal the cans perfectly without any danger of lead poison affecting the contents. There are times, however, when these machines get out of order and do not work smoothly, and great care must be exercised to avoid contaminations from soldering solution and small drops of solder. The latter often get into the cans from the "tipping" or "dotting," which must not be done until the caps are cooled sufficiently. From my experience, it is a wise plan to chill the cans with air from the blower or to keep the tippers at least fifteen feet from the capping machine.

The Calcium System is manufactured by The Sprague Canning Machinery Company, of Chicago.

For processing, some prefer the regular steam retorts, some use the calcium and oil systems, and for open bath sterilization simply the open retorts filled to a certain height with boiling water, or the continuous system where the cans travel through water. These systems are all good for certain lines, but the continuous calcium system offers some advantages, from the fact that a given temperature may be maintained without the constant escape of steam. The small expense of keeping the temperature at a given point is one of the chief attractions of this system; this may be done with the exhaust steam from the engine or with only a small supply of steam direct from the boiler. After the first cost is paid the running expense is small and the cost may be saved (on fuel) in a short time.

In connection with the canning of peas there are machines used for vining and hulling which must be operated in such a manner that the shelled peas may be quickly separated and canned before decomposition sets in. I do not know of any system where the danger of depreciating the quality is so great, unless due care is exercised in handling the peas quickly. The viners are sometimes operated several miles away from the factory, and the hulled peas are put into baskets to a depth of five to eight inches; and are then hauled on wagons and piled up ahead of the separating machines. Sometimes several hours elapse before these peas are blanched and in that time the bacteria usually found on the vines and hulls get a wonderful start, producing bitter compounds and mucilaginous sub-

stances which so often cause turbidity and ropiness in the liquor of canned peas.

The machines to which we have referred are wonderful achievements of skill and practical knowledge, and if properly operated give better results than hand work, but they should always be set up quite near the factory and should not be operated faster than the peas can be handled in the canning department. Many samples of canned peas, having clouded liquor, have been sent to the National Canners' Laboratory, and the cause has been frequently traced to the improper method of operating the vining machines. The vines should be cut, hauled to the factory, and the motto should then be: *"From the vine to the can in the shortest possible time."*

The manufacturer of special food products requires special machinery, which may have to be built according to the packer's ideas and for the accomplishment of special requirements. There are a great many special food products sold under trade names, and the machinery for such will be described under the particular headings. One important fact must ever be borne in mind, viz.: Every package will reach a customer, and if there are any defects due to carelessness on the part of employees who operate the machines or to break-downs, they should be watched and the cans should be inspected at the point nearest to the final sealing, and all that are defective in any way should be taken out. Every can either makes a friend or an enemy of the consumer, and the proper handling of the equipment is therefore very important.

Capacity of the equipment is another important factor in producing goods of fine quality. There should always be enough machines to take care of all the raw material received as rapidly as possible. It is not wise to run full with no reserve machines to take up the work in case of break-downs. This point is frequently overlooked by some packers and it happens most frequently that the capping machines are unable to take care of the cans. A reserve machine should always be kept in good running order, so that any breakdown may not delay the cans which are waiting to be sealed.

It is not a good plan to operate too many automatic machines in a single system. Automatic machines frequently get out of order, or need adjustment of some delicate parts, and if a number are operated in a single system, there is too much loss of time.

It is better to operate some machines, from separate shafting, and regulate the speed in such a manner as to keep the whole system in running order.

Machines should never be crowded too closely. As a rule, losses and damaged goods result from overcrowding. There should be plenty of room to operate them and the most experienced people placed in charge, to get the best results.

There are many factories throughout the country which are not properly equipped. Every improved machine should be carefully studied, especially if it has any advantage in the speed and character of work turned out. Many packers, on the other hand, go to extremes and adopt every new machine put on the market, whether it offers any advantage or not, and the expression, "I am machinery poor," is frequently heard. It is perhaps a good plan to take in, on trial, any machine which appears to offer some advantages, but if there is loss of time or danger of injuring the quality of the goods it should not be accepted. Therefore the proper equipment of a canning factory is a problem which must be solved by every packer for himself. The chief points to be observed are the continuous arrangement, to avoid retracing; selection of only the best machines; the proper placing and operating, with due regard always for speed and improved quality of the goods manufactured.

WHAT TO PACK.

This is an important consideration; "what to pack" claims the attention before either building or equipment. It is sometimes difficult to foretell just what specialties may become a part of the business after it is established, but it must be decided just what will be the best line of goods to be manufactured. When the conditions are favorable, peas, tomatoes and corn are packed by some houses, but generally not more than two of these are packed in one location. In some locations the whole interest centers in one, and then other kinds of goods, such as berries, fruits, pumpkin and a long line of specialties, are packed to keep the factory in active operation and give employment to valued help.

There are certain sections of the United States where peas, tomatoes or corn are better in quality than those grown anywhere else; thus the New England states, New York and a few other places, are noted for the fine, rich flavor of the corn grown there. Michigan, Wisconsin and some other places are noted for the delicate, sweet flavor of their peas. Delaware, Maryland and Indiana are noted for their excellent tomatoes, and many other places have records for the excellency of this valuable product.

There are certain localities where a very excellent crop of any of these staples is raised occasionally, and when the report is published it sometimes proves a strong inducement for the establishment of canning factories. There are many places which boast of their canning factories and are not able to supply them with the products to keep them running.

There are certain localities where the conditions seem favorable for large crops of peas, corn, etc., and in fact large quantities are grown, but the flavor and quality in general is quite poor in comparison with that of more favored places; the peas are mealy

and almost tasteless perhaps, or the corn is tough, dry and not sweet, and so it is impossible to get a quality of the canned article that will compare favorably with that of other sections.

It is for this reason that saccharin has been so generally used by packers of peas and corn in unfavored locations. There is no question but that a small addition of granulated sugar does improve the quality, but the cost is great, and so saccharin sold under various trade names has been used by not a few packers.

In determining what to pack, it is not enough to have large crops of any particular fruit or vegetable, but the quality must also be determined to see how it will compare with that of well known standards. All the skill and knowledge possible would not raise the standard up to the very best, unless the original quality was good. How many houses are trying to do this very thing? When it is certain that the raw product is inferior in quality and that no amount of skill in packing will ever bring the finished product up to the regular standard, it would be wise to discontinue for the reason that no reputation can be built up on such goods, and even though a small margin may possibly be realized, the reputation is injured and the general effect on the market is not good. A location may be very favorable for packing tomatoes, but not for corn, so it would be better to build up a line of tomato specialities which may be manufactured during the months following the tomato season, and not attempt to pack corn, even though large crops may be realized, if the quality be not up to that of recognized standards.

Some houses make a specialty of canning tomatoes and follow this with the manufacture of tomato catsup, Chili-sauce, etc., which is a splendid idea if the tomatoes are well suited for making these condiments. It is a fact worthy of notice, however, that only a few localities produce tomatos fit for strictly first-class tomato catsup.

Much of the piquant flavor of catsup is due to the natural acidity of the tomato, and such tomatoes are not as solid and meaty as are generally used for canning purposes. As a rule the finest tomatoes for canning are solid and do not contain too much juice.

Small fruits are not grown in sufficient quantities for canning purposes outside of certain sections, such as the eastern and extreme western coast states and the fruit belt near the Great Lakes, and although other localities may at times have crops of berries and peaches, they are, as a rule, inferior in size and flavor to those grown in the places we have named. It is possible, however, to have these fruits shipped into less favored places, and they may be either canned or made up into jellies, preserves, butters and jams; but the expense of shipping is great and the quality very materially affected, therefore, it is advisable to pack such goods as near as possible to the point where the fruits are grown. One factory in California is located in the center of its orchards and the flavor and quality of the

goods packed is very fine and is a credit not only to the firm, but also to the whole industry. The fruit is packed as soon as it is picked.

There is a long list of specialties, some of which may be added to a business which will keep the factory running between seasons, and they are quite profitable, too, if the very highest quality is maintained. There are a number of concerns which produce lines of specialities to fill in between seasons, and the quality of the goods is so poor that the market is glutted, and the whole industry suffers materially.

Some of the houses of which we are speaking sent up into Michigan several years ago and bought large quantities of navy beans which were moldy and blighted on account of rains and unfavorable crop conditions. These beans were soaked, boiled and covered with a very low grade of sauce, and No. 3 cans were sold on the market for 10 cents. They were not fit to eat and the people became disgusted with this specialty in so much that even the goods of highest quality moved slowly for a time.

The same practice injures the catsup market frequently. Tomato canners will dump their tomato peelings into open-head barrels or casks and perhaps let them sour and almost decompose before they are made up into pulp and finally into catsup. It might be remarked that catsup made from such material will keep almost indefinitely without any preservative. The lactic acid formed during the decomposition of the peelings, takes the place of vinegar and preserves the catsup. Some of the strictly pure catsups mentioned in the Agricultural reports are manufactured from such refuse, and arguments against the employment of chemical preservatives in fine catsup have been built upon the facts mentioned. Fine catsup requires a preservative unless it is sterilized. The effect of such poor goods thrown on to the market injures the sale of even the finest grades, because people become dissatisfied and turn to other goods, or they make catsup at home and quit buying altogether.

There was a time when the manufacture of jellies, preserves, butters and jams was a profitable business; this was in the beginning, when all were made from the pure fruits, and there were no imitations or adulterations widely known. There was a good demand, and the profits were good, but when the imitations and adulterations became so gross that the goods had no flavor nor resemblance to the fruit from which it was claimed to be manufactured, the people quit buying the stuff and the prices dropped so low that there was no inducement to pack these goods.

Today, however, there are a number of such lines manufactured from strictly first-class material, and are pure, and there is quite a good demand for them, but the market has been greatly injured by the inferior lines and it will require time to restore confidence.

It is well when any line of specialties is to be made a part of the business, to manufacture such goods as will be harmonious with the regular line. For instance, if the canning of tomatoes is to be the principal product, the line analogous would include tomato catsup, Chili-sauce, and perhaps tomato soup and goods requiring tomato sauce or other delicacies which combine nicely with tomatoes. It is surprising how naturally the side lines have sprung out and helped make some of the gigantic institutions identified with the food industry.

The lines which have sprung out of the dressing of meats for the market are the canning of meats, soups; also extracts, oils, medicines and chemicals, and other lines seemingly to have no connection, and yet very important in the saving of material formerly wasted.

SELECTION OF RAW MATERIAL.

The selection of raw material is perhaps the most important of all steps in the art of canning and manufacturing food products. How is it possible for a firm to produce goods of fine quality unless the raw material is of the very best? To begin, there should be a thorough understanding with the growers just what variety is to be planted. Vegetables such as corn, peas and tomatoes should be grown according to contract, and it is a good plan for the packer to select the variety or furnish the seed to the farmer. If tomatoes are to be grown, the packer should decide whether he intends to manufacture catsup and similar products at the same time and furnish seed for the varieties which produce the best natural color and those which are piquant in flavor. If the packer decides to pack corn, he should select the seed of such varieties as are white and sweet, and if peas are to be canned, the varieties should be only those which are tender, sweet and those which retain their natural color well. These same principles should be applied to all products so far as possible. The next step is the contract with the growers, which should be as strict as possible, especially covering the time of harvesting and delivery. All farm products should be delivered on the same day when they are taken from the field. As we have stated in previous pages, the motto should be: "From the field to the can in the shortest possible time." Raw material which has stood over one day loses much of its flavor, and besides offers great objections from a bacteriological standpoint. If the product is wilted or softened or partially decomposed the flavor is greatly injured and the liability to spoilage is increased. We have shown how necessary it is to increase the time for sterilization in this case, and this means additional loss of flavor, so that the result of it all will be only inferior goods. During some seasons various blights,

rusts, smuts and rots attack the raw material and cannot be avoided absolutely, through our present inability to cope with the fungi and molds which are responsible; but we are able to cut away such portions and use only the good parts. Tomatoes and cabbage are liable to black rot, apples are sometimes attacked by fungi, also other parasites, and if these parts are removed the good parts are equal in flavor to sound fruit so far as I have been able to determine, because the disease is only local and does not affect the whole fruit unless, of course, it has advanced too far. Whenever possible, however, only perfectly sound material should be used, but it sometimes happens that these diseases cannot be avoided and the best has to be made of the matter.

In selecting all ingredients which enter into the various formulas for making special food products, the very best are to be used always. It may be necessary to make analyses of some to determine their purity and the presence of colors and antiseptics, and a careful study of the official analyses given in Chap. IX will be found serviceable. Wherever possible the packer should familiarize himself with every detail of his business. He should know the chemical composition of the fruits and vegetables he cans, also the food value of each, and should study the effect of various degrees of heat on the nutritious properties. Of course this is impossible if the business is large, and in that case he must employ men who are able to investigate all practical and scientific problems relating both to raw materials and the finished goods.

CHAPTER XII.

Peas

History, Growing, the Leguminous and Nitrifying Bacteria, the Pea Parasite, Chemical Composition and Food Value of Peas, Methods of Canning, Machinery, Bacteria Associated with Spoilage, as Found in Various Actual Losses.

HISTORY.

The garden pea belongs to botanical order of Leguminosæ, in the sub-order Papilionaceæ, and family Sativum. The origin of peas antedates all written history, since early records show that they were common at that time in the East. Holland seems to have been the first European country to cultivate this variety of pulse during the middle ages, and from that country they were introduced into England, and then the seed peas were probably brought over to America by the pilgrim fathers. There are two kinds of peas, which are separated botanically into distinct families, viz., the field pea, cultivated as feed for cattle, and the much esteemed garden pea,; but it is probable that, originally, these were the one species, the latter having undergone marked changes under special care of horticulturists, until it now yields a highly-prized article of food.

The flowers of the field pea are red and only one to each flower-stock, while those of the garden pea are more commonly white, seldom red, and there are two or more to each flower-stock. The pea vine is a climbing annual, having pennate leaves, ovate leaflets and branching tendrils.

There are many varieties of garden peas cultivated especially for canning purposes, and these are selected with due regard for small sizes, sweetness and flavor, since the small sizes are in great demand and bring the best prices. There are, however, large sizes of the wrinkled variety, which are very sweet, and have a good market at all times. One variety often grown for the market, because of its excellent yield, has black eyes and very little flavor. When canned these peas have a mealy, slightly bitter taste, and cannot compare in any way to such varieties as the Little Gem, Alaska, Admiral Advancer, Horsford's Market Garden and others.

The canning of peas originated in France some time between 1810 and 1820, under Appert and others whose names are not known to us. Such excellent peas have been cultivated and canned

in France that their reputation is almost world wide; but in late years there has been too much artificial coloring with copper salts, and there is considerable objection to them on that account. The French, however, give more attention to the cultivation of peas than Americans, and have as a result a much larger per cent of small sizes, which are sweeter and more palatable than the larger and more matured sizes.

In America, pea packing began about 1860 in Baltimore, and the demand became so great that the industry was almost unable to meet it, although every canning house in the country packed them wherever the location was favorable for growing them. The labor connected with picking peas in the fields, and hulling them at the factories, was enormous, and this expense naturally made the retail price a little too high for the masses; but in 1890-1892 Messrs. Chisholm & Scott overcame the difficulties of hulling by machinery, and by wonderful genius, machines were perfected, which no longer necessitated hand work either in the field or the factory, so far as picking and hulling were concerned. From this time until the destructive pea louse appeared and devastated the crops of peas in many places, the canning of this popular vegetable increased wonderfully, even to the extent of over-production.

All varieties of peas were grown and in localities which were unfavorable the peas did not have a good flavor, so that the quality of a certain per cent of those packed in 1891 and 1892 was not strictly first-class. As we stated in the last chapter, there are only certain sections of the country well suited for cultivating peas. The flavor of these is due to several causes; the climate is such that the growth is rapid, consequently the peas are very tender and sweet, the soil is particularly adapted to the development of the nodules or legumes, which are excrescences from the roots, and have the power of fixing the free nitrogen, which is then used by the plants themselves. These nodules are bacteria, and are termed *nitrogen-fixing bacteria* and *bacteroids*.

The nitrifying bacteria also assists in the formation of nitrogen salts, which are used by the growing plants, so that soil which contains large numbers of these microscopical organisms furnishes the best possible condition for the growth of peas.

Peas grow well in chalky and other calcareous soils, but a fine growth depends almost entirely upon the presence of bacteria we mentioned, and it is possible to prepare the conditions artificially, which will secure a fair yield even in most unfavorable locations, by making pure cultures of these bacteria and mixing them with the seed when planted, so it is probably well that we make a closer examination and study of these minute organisms which are so useful.

ASSIMILATION OF NITROGEN.

The sources of nitrogen for the use of plants are: The atmosphere (which contains about 79 per cent by volume); the nitrates and nitrous acid formed in the soil and air; ammonia (produced by the putrefaction of dead matter); manure and fertilizers, which contain nitrogenous compounds; and from the tissues of plants and animals. Plants cannot of themselves use nitrogen in free form, so it must first be fixed in combinations suitable for its assimilation. The value of fertilizers lies in the amount of nitrate of soda which they contain, and this salt is found naturally in Chile and Peru, South America, where it has accumulated for hundreds of years by the nitrogen-fixing bacteria, which use the free nitrogen of the air and combine it with soda, so plentiful in those regions. These nitrate beds are being exhausted rapidly, however, nearly one and a half million tons being exported annually, the value being about $65 a ton. Nitrate of soda is used in such large quantities in various industries that the supply for fertilizing is growing less, and it is estimated that within fifty years the natural beds will be exhausted; so some other means of obtaining the salt for plant life is a problem which is open for solution by every one. Since the discovery that certain bacteria have the power of utilizing the free nitrogen of the air, and are able to fix it with soda, it has been discovered that certain plants have a tendency to form legumes or tubercles upon their roots, and that these are nothing more or less than nitrogen-fixing bacteria, which supply the plant itself with the nitrogen elements, so that the bacteria themselves are used by the plant and are its hosts, contrary to the general system seen in nature, where the breaking down of animal and vegetable protoplasm is a source of life and energy for the development and growth of fermentative, putrefactive and parasitic micro-organisms.

The green pea is one of the species of plants which invites the nitrogen-fixing bacteria, and at first furnishes them the elements necessary for their growth, afterward claiming them for its own existence and building up a healthy stem with flowering branches, gives evidence of its well nourished condition in the well filled pods of tender, sweet peas, so earnestly sought by all lovers of this fine flavored garden pulse.

When peas are planted in a soil containing all the elements for growth excepting nitrogen, they will thrive well if there are any nitrogen-fixing bacteria present, because these little workers will build up the nitrates for the plants, but if none of these are present the growth is poor, because they must feed upon the carbohydrates, albumen, fat, etc., accumulated in the seed-leaves, so when this supply is exhausted the plants cease growing, and the leaves lose their chlorophyl, turning yellow, and there is perhaps no disposition to

bear the pods. The plants in this case are suffering from what is termed *nitrogen hunger*. When at this stage, if the soil near the root be moistened with water containing the nitrogen-fixing bacteria, wonderful changes will be noticed in a short time. The stock and branches will grow stronger, the leaves will turn green, and the pods will fill with peas rapidly. In order to properly grow peas in some localities where very poor or only occasional crops are obtained, a means is now offered to the packer of helping the growers to obtain good crops at all times, and this discovery is of course valuable to canners, because they must, to a certain extent, be guides for the farmers, who do not, as a rule, keep posted on scientific researches in agriculture.

Plate 99. Nitrogen Fixing Bacteria

Photomicrograph X 1,000. Bacillus Radicicola, rod forms cultivated on special agar mitrient medium. Stained with Fuchsin.

In 1888 Beyerinck made the discovery that the nodules of tubercles on the roots of plants belonging to the order of Leguminosæ were composed of bacteria which had changed from simple motile rods into a complete involutionary form, having no resemblance to the original micro-organisms whose protoplasm had been used to build up bacteroidal tissue. He obtained pure cultures from the nodules, and used the leaves of the plant with the addition of 7 per cent gelatin, ¼ per cent asparagin and ½ per cent of cane sugar, as a solid culture medium for isolating them.

To cultivate them in pure cultures, we take a nodule from the roots of the pea and wash it with water, then steep it in absolute alcohol for about two minutes, and then drive off the alcohol with ether. The nodule is then cut open and a portion of the bacteroidal tissue is introduced into a small quantity of water, which has been sterilized in a cotton-plugged test tube at 250 degrees F. for twenty

minutes. After the infusion has stood a while, the Petri dishes containing the nutrient medium previously described are moistened on the surface with drops of the infusion; the gelatin will absorb the water and leave the germs free on the surface. These will develop and form small mucinous colonies, which do not liquefy the gelatin, and streak cultures may be made from these. Two varieties of micro-organisms are thus separated, one which has been named *Bacillus radicicola rods,* is about 1 μ broad and 3 to 4 μ long. They are strongly aerobic and may be seen to seek the air bubbles under the coverglass or to wander towards the edge of the glass. The other variety, called *Bacillus radicicola rovers,* is exceedingly small, being only about 0.9 μ long and 0.18 μ broad; they are motile, possessing a single terminal flagellum, which is about twenty times as long as the cell, and gives it a very rapid movement, which enables it to break away from the parent colony and travel rapidly across the surface of the gelatin. The germs are so small that they readily pass through the Chamberland filter and escape ordinary notice in stained coverglass preparations.

The author is indebted to George T. Moore, physiologist in charge of Laboratory of Plant Culture, Bureau of Plant Industry, for his first cultures of these organisms, but we have obtained even better cultures for peas from the young nodules previously described, and pure cultures are now grown in the National Canners' Laboratory and these are free to all subscribers for distribution among growers whose ground seems unfavorable at times for producing good crops of peas. It is possible to take a dry culture containing millions of these germs, and inoculate a quantity of rain water specially prepared with nutrient material, and after a few days soak the seed peas in the water, and plant them, or the manure that goes into the field may be moistened with such water, and the bacteria may thus be distributed over a field in such a manner that the crop of peas will be twice as great as is ordinarily grown on the same ground.

The bacteria which prove so valuable in fixing the atmospheric nitrogen for the benefit of peas, have a peculiar life history. They are widely distributed, in the air, water and soil, but are frequently absent in some localities, or if not entirely absent, are so few in numbers as to be of little value to the peas sown in such places. If the seed peas be moistened in water, which has received a pure culture, they will be carried into the ground and will be able to grow and multiply rapidly as soon as the tiny hair-like roots begin to force themselves downward into the soil.

The roots absorb the moisture from the soil, and through the epidermal cells, the bacteria gain entrance and rapid multiplication takes place by the fission process, so that in a short time the sap

is teeming with countless myriads of these tiny organisms, which fill up all the channels, multiplying until this cycle of their life history is accomplished.

The bacteria are seen to be gathered into colonies in various places where hard membranes surround them, and sacs are formed which grow outward and beyond the bark cells of the roots, so that tubercles or nodules are formed and these become hard and present the appearance of tissue. The tissue is formed by the bacteria themselves, which no longer have any of their original characteristics or forms, but are matted together into bacteroidal tissue, which

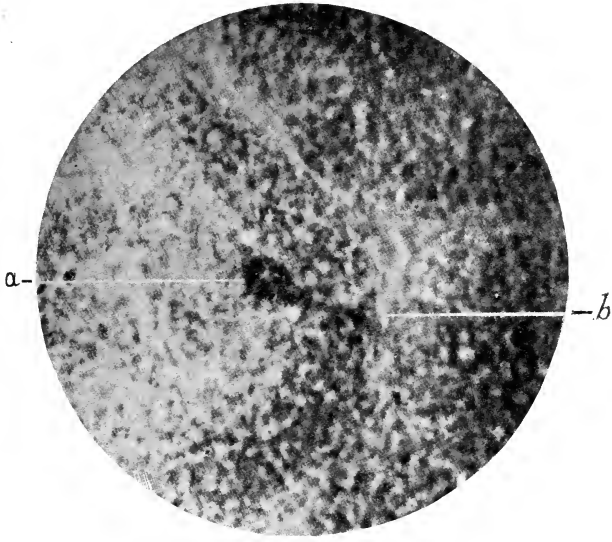


Plate 100. Nodule Bacteria Radicola

Section of a Nodule, a Shows Cell Containing Bacteroids, b Shows the Infected Thread, Photomicrograph X 1,000.

is called by A. B. Frank, mycoplasma. The name which has been given to these cells is "bacteroids," which indicates their origin from bacteria. The bacteroids are rich in albumen, and never again grow into the rod forms, seeming to have entirely lost reproductive power. The accompanying plates will give some idea of the changes which the bacteria undergo from the rods to the formation of the nodule tissue.

It will be noticed that slight swellings appear at the ends, which begin to divide into branches or forks, later taking on distinct Y forms, which unite and form a meshwork, or reticulated bands. Within this meshwork are frequently found rod-shaped bacteria which seem to have escaped the involution which has affected others of their kind, and these bacteria may be employed to start pure cultures on specially prepared nutrient media.

According to A. B. Frank, the nodules formed on the roots of peas, differ from those of other Leguminosæ, in chemical composition, and contain besides albumen, carbohydrates, which he names *amylodextrins;* but other investigators have shown that the socalled amylodextrins were fatty substances.

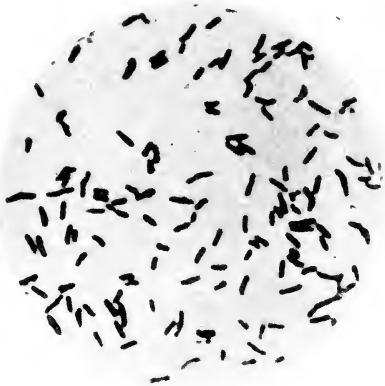


Plate 101. Bacillus Radicicola

Showing the Young Rods Attained from a Root Nodule. Photomicrograph Magnified 2,000 Diameters.

Plate 102. Bacillus Radicicola

Showing the Beginning of Involution, Clubs and Branches in Y shapes. These Forms are not Motile. Photomicrograph X 2,000.

As the tubercles grow older they increase in size, and contain larger amounts of nitrogenous substances which are utilized rapidly by the peas, resulting in abundant stalks, leaves, flowers and pods containing the green vegetable coloring matter called chlorophyl, so much esteemed for appearance in canned peas. The chlorophyl, therefore, is most abundant where the peas are well supplied

with nitrogen from the root nodules. The accompanying Figure No 20 will show the young nodules clinging to the roots and cross sections magnified to give some idea of their appearance.

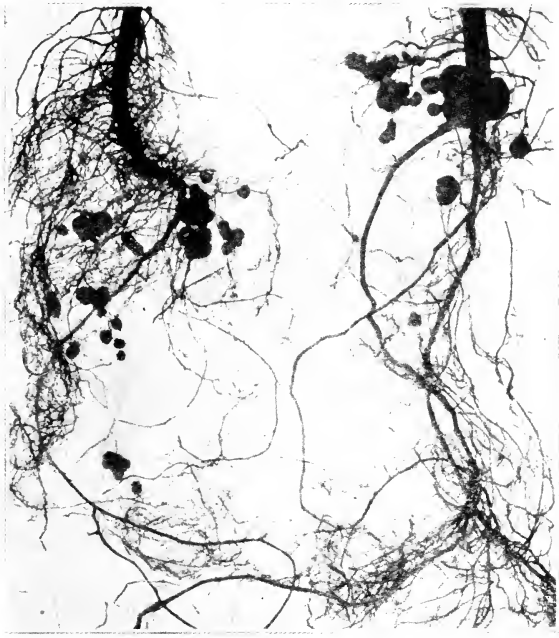


Plate 103. Nodules on the Roots of Peas

Showing Many Nodules. From Photograph in Year Book of Department of Agriculture.

J. Stoklasa has made a number of quantitative analyses of dried roots from peas, clover and other Leguminosæ, to determine the amount of nitrogen present, and the results are here given.

Nitrogen Content in the Dry Matter From	At Flowering Time.	At Fructification	In Fully Riped Pods.
Root Nodules	5 2 per cent.	2.6 per cent.	1.7 per cent.
Root free from Nodules	1.6 per cent.	1.8 per cent.	1.4 per cent.

The nitrogen is combined in three forms principally in Albumen, and also in Amides, and Asparagin, as follows:

Percentage Content of the Dry Substance of the Nodules.	In Nitrogen as		
	Albumen	Amides	Asparagin
Flowering time	3.99 per cent.	0.35 per cent.	0.34 per cent.
Ripened pods	1.54 per cent.	0.15 per cent.	traces

Other investigators have obtained as high as 6.94 per cent of nitrogen from the dry nodules of peas.

The bacteria found in the nodules of various species of Leguminosæ, look very similar under the microscope, there being no apparent difference; but experiments have proven that there are great differences in results obtained with various pure cultures.

Plate 104

Photograph of the roots of pea vine showing the formation of the bacteroidal nodules. These peas were soaked in water inoculated with Bacillus Radicicola and there are numbers of nodules which gather the nitrogen from the atmosphere and fix it for the plants. The vines are very hardy, standing over three feet high, having stems as thick as a lead pencil.

Clover planted in sterilized earth and watered with an infusion of the bacteria cultivated from the tubercles of peas, does not form nodules, consequently grows poorly, withers and dies, and the reverse is also true. If peas planted in the sterilized soil be watered with an infusion of bacteria from the tubercles of clover roots,

they soon dry while if the bacteria are taken from the tubercles of peas, the growth will be luxuriant under favorable conditions. This great similarity of species is not confined to the nitrogen-fixing bacteria; in our investigation of spoilage in canned vegetables, we have met many species which so closely resembled each other as to deceive us, except in the products elaborated by them when growing under certain influences.

The nitrogen fixing bacteria are soon to receive considerable attention in our Agricultural Stations, and some important results may be expected in the near future. The very fact that organisms which are capable of working up atmospheric nitrogen into nitrogenous compounds so necessary for plant life, opens up a means of preparing barren wastes for the cultivation of all kinds of fruits and vegetables.

Whenever crops of any plants belonging to the order of Leguminosæ are sown, and nitrogen fixing bacteria are introduced in pure cultures, there is a wonderful increase of nitrogenous compounds accumulated in the soil, which enriches it with all the requirements of plant life in general. This is the reason that ground sown in clover is so greatly benefited for the cultivation of farm and garden truck in following seasons.

There are two other species of bacteria present everywhere in all kinds of soil, which have a wonderful field of usefulness in supplying food for plant life, and their work is oxydation of the nitrogen from ammonia, into nitrous acid by one class, and then further converting this into nitric acid by the other, thus nitrites are formed from ammonia, and nitrates are formed from the nitrites, so they have been significantly named—*nitrifying bacteria.* These two classes of bacteria are always present side by side, because the elements necessary for the multiplication of the nitrate bacteria are formed, of course, by the nitrite micro-organisms. The value of manure and decomposing animal and vegetable matter as fertilizers lies in the amount of nitrogen which these substances contain, but this nitrogen cannot at once be utilized by plant life, consequently the oxydation processes we have described above, must be accompanied by the nitrifying bacteria.

The discovery of the nitrifying bacteria was made (in 1888) by S. Winogradsky, a Russian investigator, who made pure cultures from soil obtained in various parts of the world, principally from Europe, Africa, Japan, Java, Brazil and Quito (Ecuador).

The soils from these countries contained different species which he was able to isolate and grow in pure cultures, not, however, on the usual nutritious culture media employed in ordinary bacteriological work, because the nitrifying bacteria are prototrophic

strictly, and must be supplied with material suitable for their development.

A fluid culture medium was employed as follows:

Water	1000 c. c.
Magnesium Carbonate	0.5 grams
Magnesium Sulphate	0.3 grams
Diabasic Potassium Phosphate	0.2 grams
Sodium Chlorid	0.5 grams

A solid culture medium may be prepared with these ingredients using colloid silica, instead of the ordinary gelatin or agar.

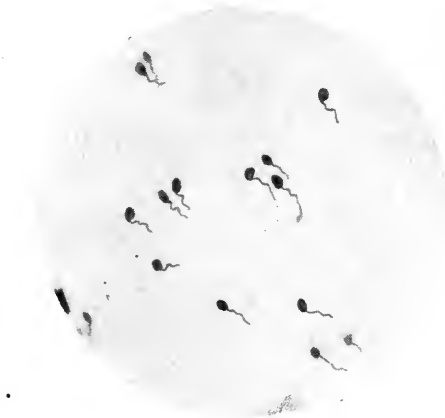


Plate 105. Nitrosomonas Europea

Showing Flagellum at end of each Germ. Photomicrograph. Magnified 1500 diameters.

NITROSOMONAS EUROPEA, is nearly round, being about 0.9 to 1 μ broad, and from 1.2 to 1.8 μ long, and is found in European, African and Japanese soil. It is a nitrite bacterium, and motile, possessing a single rather short flagellum. It grows in short chains of three or four members, and no spore formation has been observed. The colonies on silicic acid media, are brown.

Nitrosomonas Javanensis, another species, is almost round; is motile, having a single flagellum about 30 μ long, which is the longest organ of locomotion I have ever seen, being some sixty times longer than the cell itself. It grows well on silicic acid medium, and the colonies are similar to the bacterium previously described. It forms zooglea in liquid cultures, and collects on carbonate of magnesium crystals in slimy masses, and disintegrates them. After twenty-four hours the germs all drop to the bottom, and the production of nitrites ceases.

The nitrate bacteria are the organisms which form nitrates from the nitrites which result from the action of the nitrosomonas just described. They are exceedingly small, and somewhat pear-

shaped in form, and are able to pass through the pores of the Chamberland filter.

Winogradsky discovered the nitrate bacteria in the soil always where the nitrite bacteria were found, and he gave them the name of *Nitrobacter*. They are not motile, and in liquid cultures form a thin mucinous skin which clings firmly to the floor and walls of a culture flask. No spore formation has been observed, and no division of species has been attempted.

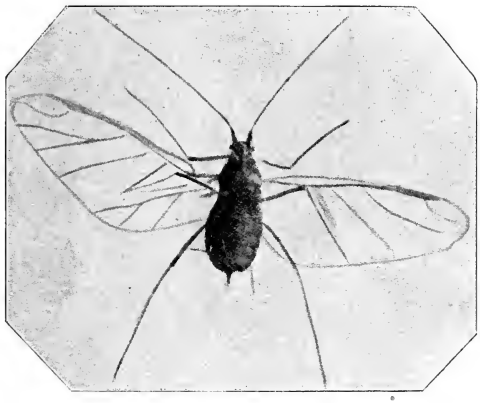


Plate 106. Green Pea Louse

There are great possibilities in the cultivation of peas in unfavorable locations, and it is possible that much larger and better crops may be grown in localities where even a fair yield is now obtained. There should be extensive experimental work done, with soil inoculated with pure cultures of nitrogen-fixing bacteria and the nitrifying bacteria. It is possible for the packers to obtain pure culture of these bacteria from the laboratory and distribute them among their growers. If these experiments are properly conducted, we predict that a very large, quick growth of peas may be obtained, and that they will be more uniform and more tender and better in color than those generally grown for canning purposes.

The experiments with nitrogen-fixing bacteria have been carried on by the Agricultural Department in the Bureau of Plant Industry for some time by George T. Moore, and the results are encouraging.

It is not expected that the packers will be able to pursue the study and cultivation of these organisms. but if they become interested, as they should, and willing to bear the expense of experimenting, it is almost certain to bring good returns for the outlay.

There are various methods for cultivating peas for canning purposes which all growers follow, but it is not within the scope of a work of this kind to enter into any elaborate description of agri-

cultural subjects. The employment of scientific methods for obtaining good results must ever be interesting to the packer as well as the grower. It may be possible to employ bacteriological methods for the extermination of such parasites as the pea louse and other insects which are so destructive at times.

PARASITES OF THE PEA PLANT.

*There are some parasites which thrive on the leaves, pods, and stems of peas belonging to the fungi. Among these are the smuts, which produce black patches composed of micro-organisms of a higher order than bacteria, but which belong entirely to the vegetable kingdom and multiply at the expense of the plant tissue and sap. The rusts are also of the same order, and frequently attack the pods so that large round spots appear all over them, and sometimes the tissue is perforated so that bacteria gain access to the peas themselves.

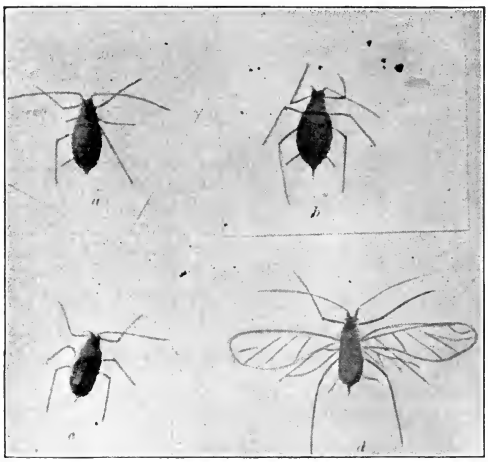


Plate 107. Green Pea Lice

A. fourth stage wingless femade; B. wingless viviparous female and young; C. pupa; D. winged viviparous female.

The damage done by these fungi is not very serious, however, in comparison to insect parasites. The green pea louse (Nectarophora pisi Kalt) or green fly (so styled by Prof. H. G. Johnson of the Maryland Agricultural College), which made its first appearance in 1899, has done more damage than all other pests known to canners. The destruction of peas in 1899, 1900 and 1901 prob-

*The photographs shown on this subject were taken by Prof. Sanderson and Prof. Johnson, to whom we are indebted.

ably amounted to several millions of dollars. The destruction of peas was greatest during the season of 1899, particularly of all late varieties. The whole Eastern section of this country and Canada, wherever peas were grown, suffered almost total losses; fields embracing a hundred acres were devastated as if by fire; so rapid was the multiplication of the insect that it proved utterly impossible to save the crops by any spraying or brushing methods known. The common method of planting by sowing broadcast had much to do with the difficulty of combating the progress of the scourge, because there was no room between the vines for brushing and spraying with kerosene, and soap-suds proved ineffective for the same reason, and, also, because the lice bred upon the underside of the leaves or between the folds in such a position that a spray could not reach them.

The life history of the green pea fly is not as yet complete, but, according to Prof. Johnson, and Prof. J. G. Sanderson (who read papers at Detroit, Rochester and Milwaukee), it seems certain that the insect is common as a parasite of all plants belonging to the legumenosæ, such as peas, beans, clover, vetch, et al., and may have been growing unobserved for many years without having attracted any particular attention. I had a most remarkable illustration of its reproductive powers on beans, (navy pea beans), which were piled in sacks. In some manner, the bottom layers became wet and were not discovered for about a month. The bags had rotted and the beans had been attacked by this identical insect, which destroyed fully fifty per cent. We swept up fully a barrel full of the dead flies which are about 1-32 of an inch long. I also found a few live ones, but nearly all seemed to have perished most unaccountably. The cause of their destruction I found to be due to a fungus disease and will speak of it a little further on.

The lice are hatched from eggs after the cold weather is over, and from that time on until winter, reproduction is carried on by the mother flies giving birth to living young. The average life of a female fly is a little more than one month and during that time she will bring into existence about 150 young ones. According to the observation of the two gentlemen previously mentioned, there are no males among them, although two or three instances pointed to their existence. There are, of course, stages in the life history of the green fly, when the males are in evidence, most likely some time in the fall of the year, at which time it is possible that the females are made capable of producing young which in their turn receive the vital principal from the mother. The many peculiarities of this kind familiar to the entomologist do not cause much surprise, because nature has endowed many species of insects with wonderful powers of reproduction, and other peculiar morphological and biological characteristics.

It has been demonstrated that cold weather does not kill the insect which may be frozen stiff, but after thawing will be as active as ever, and is still able to give birth to its young. In certain sections of the country, after having been visited with freezing weather in December, the insects were found alive after a thaw, and some were still observed as late as January on clover.

There are certain seasons when insect pests of various kinds arrive in different parts of the world in innumerable hosts, and devastate vegetation, but after a time disappear in a manner almost as unaccountable as their appearance. Nature has endowed insects with wonderful reproductive powers because of their many enemies, such as birds, animals, and other insects which feed upon them. It is strangely true that the enemies of insect life are nearly always present in sufficient numbers to prevent scourges, such as those which have destroyed peas during the past few years; but it sometimes happens that their greatest enemies are not present in sufficient numbers to prevent an overwhelming multiplication. We have mentioned birds, animals and other insects as enemies (of the pea lice), but the work of extermination by these is as nothing compared to that caused by parasites which spread disease among them. These parasites may belong to the order of fungi in some cases, often they are disease-producing bacteria which attack the vitals and reproductive organs of the insects, so that all the young soon die after they are born.

As an illustration of this truth, we will mention some fungi and bacteria, which are parasitic to insects such as silk-worms, bees and caterpillars.

In 1863 to 1865 the whole silk industry of France and Italy was almost paralyzed by a disease started among the silk worms, which deprived them of their power to spin cocoons. Pasteur undertook the task of discovering the cause, and found that a disease, which was called *Pebrine*, of bacterial origin, had spread so rapidly and affected the moth worms and eggs to such an extent that there seemed little hope of saving the industry. He retired from the world and began his investigations, which resulted in his being able to detect the disease in the moth. He would permit a moth to deposit her eggs on a small linen cloth, then he would crush her body in a mortar with a small quantity of water. A microscopical examination would reveal the presence of corpuscular matter perhaps, and the remains together with the eggs were destroyed. Whenever a moth was found to be perfectly free from the bacteria her eggs were carefully set aside for use and in this manner the healthy worms were again cultivated.

From the diseased eggs, pupæ and moths have been cultivated, shining oval micrococci, 2 to 3 μ long, 2 μ wide, and rods 2.5 μ

broad and 5 μ long, which are the cause of *Pebrine* and *Gattine* and are named by Lebert (Panhistophyton ovatum). Streptococcus bombycis, are oval cocci occuring in chains and pairs, which cause a disease among silkworms called (*Flacherie*), which causes them to cease feeding and become putrid.

Honey bees are sometimes attacked by a disease called *foul-brood*, which is quite common in this country. The disease attacks the larvæ, is contagious and causes them to die, putrefy, and turn dark brown in color. The cells containing diseased larvæ may be detected easily by the dark cappings. Foul-brood is a disease caused by Bacillus Alvei—rods varying in size which form large oval spores. The bacteria are actively motile.

An infectious disease called Caterpillars' Disease, has been observed among their larvæ. The bacteria causing their destruction are Cocci united in pairs and chains.

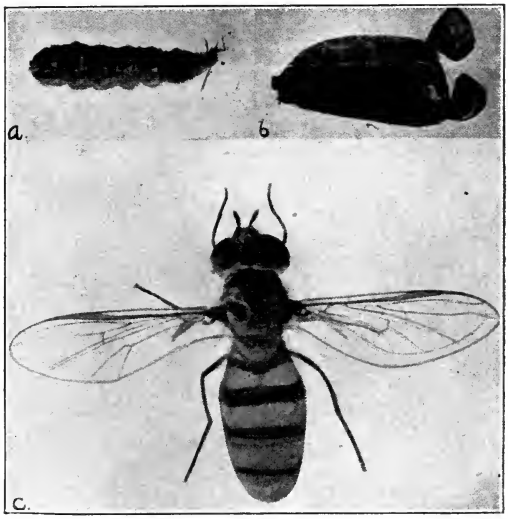


Plate 108. American Syrphus Fly

A, larva or maggot eating a pea louse; B, puparium, or pupa case, from which adult fly has emerged, end broken open; C, adult fly.

Returning to the green fly so destructive to pea vines, we find a number of natural enemies which feed upon them. There is a fly considerably smaller than the green lice, which lay eggs upon their bodies and when these hatch they feed upon and destroy the lice themselves and use their bodies as shelter until they are transformed into flies, when they emerge through openings made themselves and seek out other green lice as hosts for their eggs. Large numbers of pea lice are thus exterminated.

Even more destructive to the pea lice are the maggots of the syrphus flies, of which there are three or four varieties. These flies are beautifully banded and lay their eggs among the colonies of lice, for instinct leads them to provide a suitable food location for the newly hatched maggots, accordingly when they hatch they begin to feed upon the thriving colony of lice surrounding them, and the numbers required for nourishment very soon depletes the colony.

These maggots or worms, so often seen among the pea vines, are green or brown in color, about a half inch in length. They have no legs nor head apparently, but are provided with two powerful hooks with which they seize the lice, and hold them while they suck the fluid from their bodies.

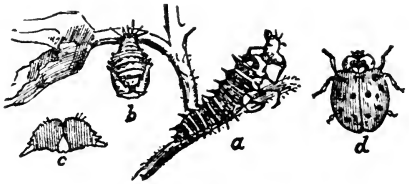


Fig. 32

The lady-bird beetles, of which there are several varieties, find in the pea lice a suitable article of diet, but since they do not come out in force until June, are a little late to be as beneficial as the maggots of the syrphus fly. The larvæ hatch out from small yellow eggs. They have six legs and attain a length of half an inch, when they attach themselves to a leaf and in a little more than a week are transformed into beetles. The larvæ and beetles both feed upon the pea lice, and consume large numbers.

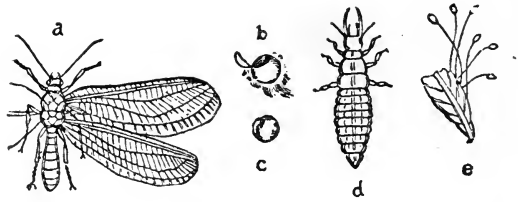


Fig. 33

There are numerous other insects which feed upon the pests, but do not flourish in sufficient numbers to materially check the spread of the lice where they have gained much headway. Among these must be mentioned the *lace-winged flies*. They are quite green in color, the shade resembling the peas.

The wings are quite beautiful, being very thin, showing the fine net work of veins which break up the sunlight into prismatic colors.

They are sometimes termed the "golden-eyed flies," because the eyes are bright golden color. The eggs are deposited on the leaves singly on a stalk of fine silk, which prevents the larvæ from feeding upon the unhatched eggs which they would surely do. Often the larger ones will feed upon the weaker, unless sufficient food is available. They move quite freely and consume large numbers of lice, grasping them in their two curved jaws, then sucking the fluids. A single lace-winged fly will lay about fifty eggs a day and the hatched larvæ one week later are busy with the lice.

By far the most important enemy of the green pea louse is fungous disease which is microscopical. This fungus is brown mold called *Entomopluthora aphidis*, and almost covers the bodies of the

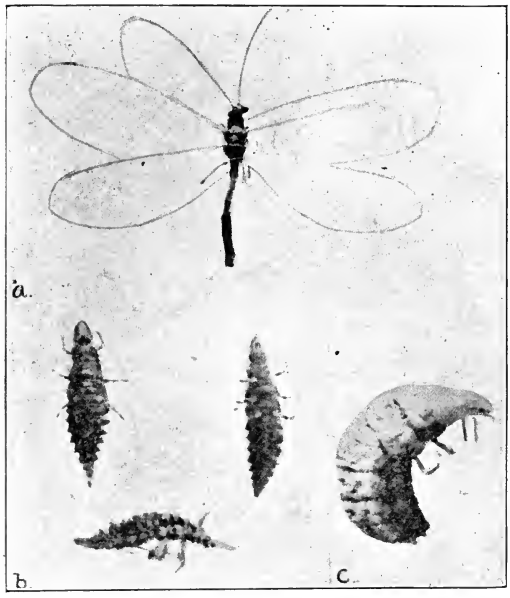


Plate 109. Lace Winged Fly

A. adult fly; B. partly grown larvae; C. pupa.

lice. It enters the tissue, absorbing the moisture, and causes the insects to shrivel up and die. The disease is contagious and spreads rapidly, carrying off more lice than all other enemies combined. It is safe to say that fungoid diseases among insects are the means of preventing their spread, since they breed so very fast that ordinary insect enemies could hardly keep pace with them. But as soon as a disease breaks out the organs of reproduction are deprived of their functions and the check is generally complete. Some of these fungous diseases are difficult to cultivate artificially, but there is surely

a method which can easily be discovered, since all the conditions necessary for the preparation of nutrient media are obtainable. This is a field for investigation and we believe it both possible and necessary for research work to be done, to check any such spread of insects as was experienced by growers of peas in the three years so well remembered by us all. This research work may be carried on, and means discovered for cultivating the disease fungi in large numbers on artificial nutrient media. When cultures are obtainable water may be used to carry the spores into all parts of fields where the lice are flourishing and thus spread disease among them, which means quick extermination.

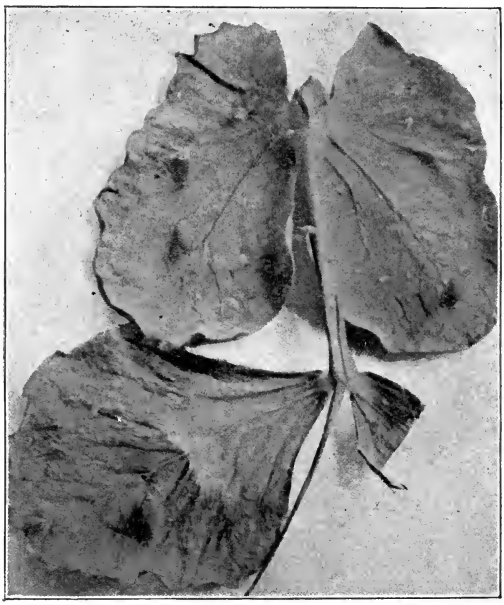


Plate 110

Eggs of Lace Winged Fly and Pea Lice Killed by Fungous Disease.

There is no doubt but that in certain seasons the weather conditions are such that disease molds will not grow naturally, but if cultures are kept in our laboratory from one year to another, we have the means of starting the disease among the lice ourselves, and need not depend directly upon nature. There is considerable expense connected with research work of this kind, but its importance is so great that packers and those interested in growing peas should take a lively interest in lending all necessary support.

CHEMICAL COMPOSITION AND FOOD VALUE OF PEAS.

Few people realize the great nutritive value of peas. We have mentioned in preceding pages that peas belong to the order of *Leguminosae,* and are included under the name of *Pulses,* to which belong also pea beans, and all other varieties of beans, lentils, and pea-nuts. These varieties of food are extremely rich in nitrogen, which, as we have mentioned, is due to the absorption of nitrogen from the atmosphere through the agency of minute organisms which have the power of fixing free nitrogen and reducing it to nitrates, which are soluble elements, quickly taken up by the plants belonging to this group. This is accomplished, as we have described, by bacteria which form the nodules on the roots of the plants, so we would naturally expect the fruit of these plants to be veritable "store houses" of nitrogen. Almost all of this nitrogen is in a proteid form, and by Church's analyses there is only 3 to 5 per cent of the total nitrogen which is not in proteid form, and on account of this characteristic the vegetables belonging to family of pulses have been named "the poor man's beef."

The proteid so valuable as a food in peas is *legumin,* which is a casein-like substance resembling the casein of milk. Sulphur is a large ingredient of the proteids of peas, and gives rise to sulphuretted hydrogen, especially when fermentative and putrefactive processes are carried on by bacteria. This gas is also liberated during digestive processes, probably due to the presence of putrefactive bacteria in the intestines. The presence of potash and lime in the composition of peas and other pulses sometimes causes calcification of arteries among strict vegetarians.

The green garden pea is particularly rich in carbohydrates, and some varieties have a very large per cent of sugar; other varieties contain very little, which has tempted some canners to use saccharin. We have explained this custom under the head of food preservatives and their detection. There is frequently a loss of carbohydrates in the blanching and processing of peas, and the addition of granulated sugar really adds to their food value.

Since peas contain so little fat they are really improved by the addition of pure butter, and I throw out this hint, which may prove valuable to any packer who is searching for specialties. We all know that pork or bacon adds wonderfully to the taste, flavor, and nutritive value of beans, and peas contain less than half as much fat as beans, consequently the nutrient value may be greatly increased by the addition of a fatty substance, such as butter. We find that canned peas heated and spread with butter are delicious, or if the liquor is prepared with butter and pepper and then poured over the peas, they are very greatly improved in flavor. The addition of cream or milk also serves the same purpose, and supplies the

necessary fats in a digestible form, a preparation much esteemed by many persons. The addition of butter to canned peas before sterilization is practicable, because the same sterilization which is required for peas will also answer for the added butter; but milk would require a stronger process, which would cook the peas to pieces, and the result would be something like pea soup, instead of canned peas.

The blanching of peas before filling into the cans is a most important feature of their preparation. In the blanching bath the slimy products formed by bacteria are washed away, and besides this a bitter substance, which is a natural component, is dissolved and freed from the peas. This bitter principle is easily detected by tasting a finely divided raw pea. Just what it is I am unable to say, but it is comparable to the principle found in aloes, quassia, and other bitter vegetables and barks. The same bitter principle is a common product of several species of bacteria, some of which have been isolated from bitter canned peas. Frequent changing of the water used for blanching is advisable, because this bitter principle is dissolved from the peas and although it is volatile to some extent, will, in time so affect the water that the blanching will not be effective.

The blanching process has some disadvantages, too. There seems to be no way of avoiding the loss of a certain per cent of proteid and mineral matter, also sugars, but the loss is inconsiderable. There is more loss of these substances from dried peas, which are sometimes put to soak for several hours. Such peas are made up into special food products, the formulae being proprietary.

Of course, the same loss is explained in the preparation of "soaked canned peas," a brand which should never be manufactured, because they are extremely poor in quality and their presence in the tra le injures the pea-packing industry.

To show the composition of peas in comparison with some other pulses I have prepared a table of analyses of solids. Of course, the great bulk of the raw and cooked product is water, which in peas amounts to 78 to 79 per cent, and in haricots and scarlet runners to 75 and 91 per cent respectively.

ANALYSIS OF THE SOLIDS.

	Fat	Proteid.	Carbo-hydrates.	Cellulose.	Mineral Ash.
Green Peas	2¼	18	72	2¼	5½
French Beans	4	14	71	5	6
Scarlet Runners	3.3	20	42	31.3	3.4
Lima Beans	2.4	21.6	68	3.6	4.4

BALLAND'S ANALYSES.

	Beans		Lentils		Peas	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Water	10.00	20.40	11.70	13.50	10.60	14.20
Proteid	13.81	25.16	20.32	24.24	18.88	23.48
Fat	0.98	2.46	0.58	1.45	1.22	1.40
Starch and sugar	51.91	60.98	56.07	62.45	56.21	61.10
Cellulose	2.46	4.62	2.96	3.60	2.90	5.52
Ash	2.38	4.20	1.99	2.66	2.26	3.50

We see by these analyses that the proteid is very large, also the carbohydrates, which make peas of great nutritive value. About one and a half pounds of dry peas would supply the daily requirement of proteid for an average man, and the energy liberated, weight for weight, is greater than white bread, beef, eggs or milk.

The relative cost of peas as compared with these foods is only about one-half, taking as a standard the amount of energy liberated, bread only being excepted. Now the people do not realize these facts and need education, so the thought which suggests itself is for the packer of peas to advertise them in such a way as to attract the masses who are spending far too much money for meat, eggs and other foods to obtain the energy required for their existence. If the people receive this information there is no doubt that greater demand for canned peas will follow.

There are great possibilities for specialties in which peas may form the base. We have already mentioned pea soup and special combinations with a sauce containing butter and seasoning, and there are, no doubt, other combinations which may be tried by experimenting, and which would doubtless prove to be good sellers. This is a field for the genius, but the genius usually strikes the successful combinations by constant experimental work. Experimental work, let me say, is one of the best paying investments. Nearly all of the most profitable lines of specialties have been worked out by the firms who are constantly experimenting to obtain combinations which are attractive, both in price and quality, and strange to say the popular specialties are combinations which have as a base some well known standard article of food.

Popular specialties have been made with wheat, oats, corn, beans, etc., combined with other materials, or worked up into modified conditions entirely new, and they have become popular because they possess real nutritive value and are palatable.

There is too little experimental work done by the canner; he is solely occupied with the idea of packing a full crop of certain products, sometimes never dreaming that these very products could possibly be made into new and attractive specialties, which might yield him two or three times the profit derived from the regular standard goods.

By proper advertising and adhering strictly to fine quality the sales of canned peas could be greatly increased. Within a year there may be means discovered which will enable the farmers to grow peas of fine flavor and suitable size, even in localities where there has been little or no success with them in the past. We refer here to the means of inoculating the seed and soil with the nitrogen bacteria described in the previous pages. When these experiments are complete there will be more peas packed and many factories will be able to pack them, even in places which seem most unfavorable.

PLANTING AND CANNING PEAS.

The old method of planting peas in rows and picking the pods by hand, the pickers having to go over the same vines a number of times, has been discontinued. The old method was expensive and the results were not always as perfect as the more modern methods. Peas are now sown the same as small grain, in drills, and there is appointed a man of good judgment who visits the fields and inspects the crops regularly, keeping well posted on the progress shown. He examines the vines and when he finds that the pods are filled with peas that are about right in the average, he orders them to be cut and hauled to the vining machines promptly on wagons, much after the manner of hauling hay or straw. Of course, the planting of peas is done at different times so that all will not mature at once, which would result in either overworking the factory or the peas would get too old. Not all of the peas are fully matured in a field ready for cutting, judgment is exercised and they are declared ready when the average is the best, preferably when the small sizes are plentiful, because these bring the best prices and are sweeter and better in flavor and color than the large sizes. The mowing machines are similar to those used for cutting wheat, oats or hay, and the vines and pods are hauled on wagons, as we have said, to the vining machines, which should be near the factory.

The vining machines beat out the peas, which are received in baskets and then taken to the cleaners, where various mechanical means are employed to free them from dirt, pieces of pods, and leaves. Usually this cleaning is done by means of a blast of air which blows away these undesirable things, or suction is employed to hold back everything excepting the peas as they roll over the screens. This does away with considerable dirt and other things and the peas are quickly taken to the grading machines which are long cylinders perforated the entire length, the holes being just the proper diameter to let the particular sizes fall through, first very small and then increasing up to the largest size, and the size which cannot go through any of the holes is caught at the end. The

proper grading of peas is very important. Great uniformity is demanded by the trade, and this is sometimes a difficult matter to accomplish, if the peas are not hurried after they are cut. If there is any shrinkage, of course, peas which are too large will pass in among the small sizes and afterwards will swell, and spoil the uniformity. Some packers divide their peas into six or seven sizes, others into four or five, which is generally enough.

CANNING PEAS.

From the graders the peas should be fed on to a linen belt, which moves slowly past the girls who are employed to pick out the imperfect or off-colored peas, also small pieces of hulls or leaves which have succeeded in passing through the cleaner and frader. These girls should wear some protection for their hair, to avoid the possibility of getting any stray hairs among the peas. Such an accident is not easily forgiven if the purchaser should be so unfortunate as to find one. There should be a rule in every canning factory stipulating that all the female employees wear caps or some other protection for the hair. Accidents are not uncommon and the injury done to the whole industry is far-reaching.

Some packers prefer to have the hand-picking done after the blanching, because the blanching brings out the colors prominently, and there may be some difference in sizes, too, which will be more apparent after the peas have passed through the water. If there are any which were shrunken they will swell up to their normal size, and if any of the yellow seed varieties which are too old, the yellow color will be more noticeable after the blanching. All these things which interfere with perfect uniformity, may be corrected at this time.

There are several points worthy of consideration at this place. If the peas are right in the beginning, it is preferable to sort them before the blanching, because it is wise to fill them into the cans rapidly after they have once been partially cooked, so it should always be a matter of prime importance to see that the peas are harvested in time and then put through the viners before they become heated. The custom of some packers, who store the vines in sheds and allow the lactic acid bacteria and the spore-bearing micro-organisms to start decomposition, with its attendant liberation of heat, is altogether wrong. Such peas must deteriorate rapidly, and the sorting or picking must necessarily be done after the blanching. One packer sent me a number of samples of peas taken from various piles which were spoiled, the liquor being muddy, and in some cases the contents were entirely sour. He states that his viners became overcrowded and it became necessary for him to fill his sheds; the result was that they became heated, necessitating careful sorting after

the blanching process. There was considerable delay during the sorting, because of the many bad peas, pieces of slimy pods and leaves. He did not increase his process, but maintained his temperature at a point just sufficient for sterilizing strictly fresh peas, and the result of it was, as we have stated, a very bad lot of peas. Should it become necessary at any time to do the sorting after the blanching, do it as rapidly as possible, having a sufficient force in order to avoid the delays so common.

The old blanching method was to have a number of small tanks filled with water, and beside each a tank filled with cold water, Sulphate of copper was used by many to fix the green color, and alum was used to toughen the skin of the peas. The peas were immersed in the hot water containing the chemicals mentioned and, after about five minutes' cooking, were rinsed in the cold water, then filled into cans.

The modern method is better because the chemicals are not used and the blanching is done automatically, the peas being carried through three baths by means of worm conveyors, or spirals, which are large enough to carry the peas through the water in a steady, regular manner. The water in tank No. 3 cleans the mucinous matter from the peas, and is attached with the overflow from tank No. 2. The second tank receives the overflow from No. 1, so all are constantly flowing, keeping the water comparatively free from dirt, slime and other matter. After the blanching a cold water spray is used to give the peas firmness and to prevent them from becoming heated.

SOUR PEAS.

Sour peas in many cases are due to careless methods before the sterilizing process, and a few words on this subject at this time will serve the packer and enable him to avoid the causes. When peas get sour prior to the sterilizing process, the acidity is generally due to organisms which belong to the Lactic Acid Group, and these germs attack the carbohydrates, converting the sugar into lactic acid without the evolution of gas sometimes. $C_6H_{12}O_6 = 2C_3H_6O_3$.

One molecule sugar = 2 molecules lactic acid.

One germ is rather small, occuring generally in pairs, sometimes in chains, but most frequently in bunches or zooglaeæ forms. It is exceedingly small, measuring only 1 to 2.8 μ long and 0.3 to 0.4 μ wide. Some writers claim to have discovered spores in this shining species but the spots which are sometimes visible within the cells. They are not true spores and do not produce vegetating forms, so we call them spore-like bodies, which are destroyed at boiling temperature.

There is a spore bearing bacillus which breaks up invert sugar into lactic acid, in fact there is a large number of bacteria which produce lactic acid.

These germs are present in large numbers on all growing vegetables and universally distributed in air, water and soil. They grow well at temperatures ranging from 100 to 120° F., and the "sweating" so often seen in hay, fodder, ensilage and pea vines, when piled in heaps, is partly due to the lactic acid bacteria. There are, however, a number of bacteria which produce lactic acid, and this acid being quite sour, imparts that characteristic to any food product susceptible to lactic fermentation.

Plate 111

Photomicrograph of Lactic Acid bacteria which generally gain entrance to canned goods through leaks. These bacteria do not produce spores and are easily destroyed by boiling temperature. Cultivated from cans of spoiled corn; stained with carbol fuchsine. Magnified 1,000 diameters.

When pea vines are piled up in heaps, the "sweating process" begins quite soon, and within a few hours large quantities of sugar and starch have undergone chemical changes, with the formation of considerable lactic acid. There are usually a number of organisms at work at the same time. Some of these belong to the class of heat-loving bacteria, and by their united action on the cellulose and proteids, the temperature if often elevated to 120 degrees F., when the juices are freed, and the so-called "sweating process" is to be seen by overturning the vines.

There is no part of the process of pea-canning which affords so much danger of sour goods as the sweating of the vines and pods, so there is danger of allowing the raw material to accumulate too

Bacillus Butyricus, Hueppe

Origin.—Milk.

Form.—Long, narrow rods, having rounded ends; found frequently in pairs; may form threads.

Motility.—Actively motile.

Sporulation.—Forms bright median spores, oval in shape, at about 30°.

Anilin Dyes.—Stain well.

Growth.—Rapid.

Gelatin Plates.—The deep colonies form masses of a yellowish color, the surface colonies liquefying rapidly and then forming grayish-brown, granular patches having fibrillated borders.

Stab Culture.—It liquefies slowly along the entire line of inoculation. A thin, folded, grayish-white scum is formed on the surface, the gelatin becoming a yellowish color. The liquid remains cloudy for a while, but eventually clears up, the growth settling at the bottom.

Streak Culture.—On agar, a thick, yellow or grayish, sticky growth is formed. On potato, a light brown, transparent covering grows, sometimes becoming folded.

Milk.—The casein is gradually coagulated, as with rennet. After about eight days, the casein is redissolved or peptonized with the formation of leucin, tyrosin, ammonia and bitter products. It forms butyric acid from hydrated milk, sugar and lactates.

Oxygen Requirements.—Aerobic.

Temperature.—It grows best at 35° to 40° C. but can grow at ordinary temperature.

Behavior to Gelatin.—Gelatin is liquefied by it.

Aerogenesis.—It forms butyric acid.

Pathogenesis.—Has no effect on animals.

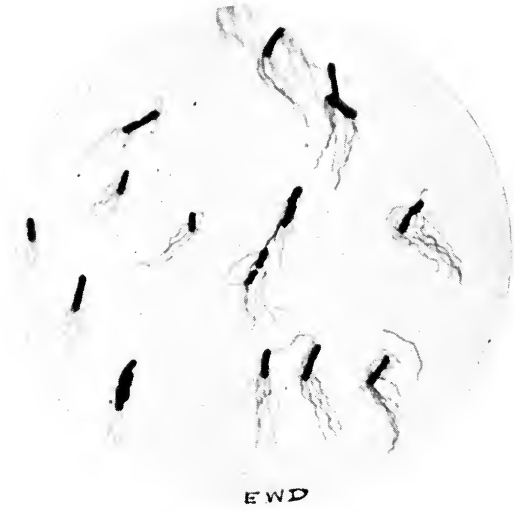


Plate 112. Bacillus Butyricus, by Hueppe

Butyric Acid Bacteria, showing Flagella, Species isolated from Peas undergoing "sweating." Stained by author's method. Photomicrograph by author. Mag. 1,200 diameters.

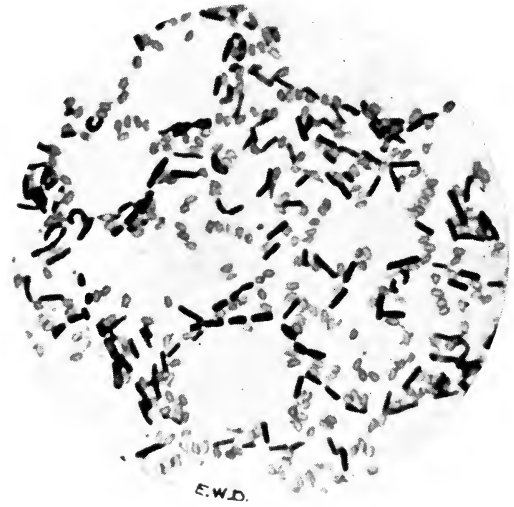


Plate 113. Bacillus Butyricus, Hueppe

Butyric Acid Bacteria, same as shown in Plate 107. Rods and Spores. Stained with Cabol Fuchsine. Spores are very resistant to heat. Photomicrograph by author. Mag. 1,500 diameters.

far ahead of the machines. A large per cent of the spoilage cases of peas investigated in the laboratory have been due to souring which happened prior to the sterilizing process. Our readers will recall the great trouble experienced from sour peas after the viners were first enstalled. I do not wish to throw any reflection whatever upon the viners, because I believe that they are splendid inventions, but the factories were not properly equipped for handling peas by this system. The packers in many cases had not planned for the increased receipts of raw material, and the consequence was that the vines were cut too fast. If the viners were operated at their full capacity, the hulled peas would pile up ahead of the other machinery, so that by the time the peas were finally sealed in the cans, they had undergone marked chemical changes, the carbohydrates or sugars having for the most part been converted into lactic acid, and in some cases bitter compounds had formed, together with butyric and fatty acids, all due to the action of bacteria of various species.

One peculiarity of sour peas is that it is impossible to tell by the appearance of the cans that they are sour; the cans appear to be all right and have the usual vacuum, which draws in the ends, and do not swell. Very often this is the case, although not always, because souring without the formation of gas may result from imperfect sterilization; but in the great majority of cases this takes place prior to the sterilizing process.

From the very fact that appearance of sour goods gives no indication of the trouble, the cans may be scattered throughout the piles and cause a great deal of dissatisfaction when they reach the trade. The question is frequently asked: "How will I be able to pick out the sour cans so that I may avoid trouble with the trade?" I will give you a few practical hints which may serve you, should you be so unfortunate as to permit your goods to get sour. Nearly all fatty acids are volatile to some extent, and if heated pass into a gaseous state, although not completely, and after cooling will again become liquid, but sometimes quite slowly; now we can take advantage of this physiological characteristic and heat our cans in boiling water until the ends of all are swelled, then by applying cold water the cans whose ends draw in rapidly are good, while those whose ends draw in slowly are probably all sour. To do this expeditiously, place the cans in crates which hold only a single row of cans, then turn the bottoms upward, then lower into open bath of boiling water and heat until the ends of all the cans swell completely. Do not heat long enough to cook the peas, because the tender peas may become too soft and dissolve in the liquor, thus making it turbid or cloudy. After the ends are all swelled, lift the crate from the boiling water into a tank of cold running water, just deep enough to be entirely submerged. In a short time the ends of the good cans

will respond to the vacuum produced, and will snap back, while those cans which contain volatile or fatty acids will remain swelled for perhaps thirty minutes to one hour. Of course some good cans will not draw in rapidly, especially if they had been somewhat chilled before sealing; the vacuum in this case would be quite weak and of course would not respond quickly to the chilling, in the process previously described. It is not always possible, therefore, to save every good can by this method, but if due care and judgment are exercised, all sour cans may be detected and removed. Sour peas cannot be made good. They are a loss and should be dumped.

The "sweating" previously described is not the only source of sour peas. There is no stage of the process where delays are so dangerous as after the blanching. Bacteria as a rule are true scavengers and invade partially cooked material rapidly. The cooking which the peas received in the blanching is attended with a certain loss of carbohydrates and proteid, and the liberation of natural juices and softening of cellulose, so they afford a rich soil for the development of bacteria. Furthermore, the heating softened the spores of the heat-resisting bacteria, which rapidly develop into vegetating rods, forms which produce the remarkable changes so often noticed in canned goods.

During any of the delays occuring after the blanching, the danger of souring is great, so it is always preferable to do the sorting prior to the blanching. Delays sometimes happen where the peas are filled into cans, and at the capping machines, where great stacks of cans are piled up. Like all automatic devices, these machines sometimes get out of order, and considerable time is lost making changes or repairs, so it is advisable to have extra machines to avoid the delays as much as possible, in order to prevent souring of the peas at these times.

The blanching of peas should vary in length of time according to size, the small peas should receive less cooking than the large sizes, the time varying from five to ten minutes.

Some successful packers use alum in the blanching to harden the skin of the peas, and I do not see any objection to it. It is hardly likely that the employment of alum for this purpose would be condemned by food commissioners as illegal, and while its employment is not absolutely necessary, nevertheless it does prevent the cracking of the skins, and will insure a much clearer liquor for that reason.

The filling of peas into the cans was formerly done by hand by the aid of small funnels, and the perfection of automatic devices for Recently, machinery for this purpose has been made which accomplishes the filling with great uniformity, giving better average results than hand filling. One bushel of peas fills about fifteen No. 2 cans, approximately.

The filling of peas into the cans is important; the cans must open up full, but care must be taken not to fill them too full because the peas will crack open in the sterilizing process.

After the cans are filled with peas, enough weak brine is added to cover them. The brine may be filled by machinery—an attachment for this purpose is usually connected with the pea filler. The brine is made by dissolving about six pounds of salt in forty gallons of water. Some packers use a small quantity of saccharin to sweeten the peas. Eight pounds of granulated sugar added to the brine gives splendid results.

The brine should be made quite clear, and where filtered water is available it is to be preferred, although well or spring water is very good. The water used for all canned goods should be pure and clear, surface water and the muddy water so often seen in large cities, which obtain their supply from rivers, is not desirable, unless properly filtered. Water which may be contaminated from sewage or decomposing vegetable matter, so often seen in the vicinity of canning factories, should not be used, because it may contain products elaborated by bacteria which may injure canned goods. Much of the salt sold on the market is impure, containing much foreign matter, so it is advisable to pay a little more and obtain the refined table salt, which with pure water ought to give a clear brine. Granulated sugar is to be preferred to ordinary light brown sugars, because it will stand the process without any burnt sugar flavor. During some seasons, and in some localities, the peas are sufficiently sweet without the addition of sugar, but ordinarily a little sugar adds greatly to the quality of peas.

MUDDY LIQUOR.

Muddy liquor is so often the occasion of losses that I will explain some of the causes which have come to my notice. The phenomenon is usually noticeable a few days after processing; in some cases it is seen as soon as the cans are processed. If we examine the peas which have stood for some time before blanching, we will notice that they are quite sticky. This viscid matter is due to the action of bacteria, and is the product formed by the growth of the germs on the carbohydrates and proteids. One of the principle agents is Bacillus mesentericus vulgatus, an organism first found on potatoes, for which reason it is sometimes called the "potato bacillus." It is, however, a widespread variety and is present in water, soil, and on the leaves, pods, stems and roots of nearly all vegetables.

Bacillus mesentericus vulgatus is a thick bacillus, measuring from ½ μ to 3.5 μ in length, straight, with ends somewhat round, actively motile when young, due to numerous flagella which grow

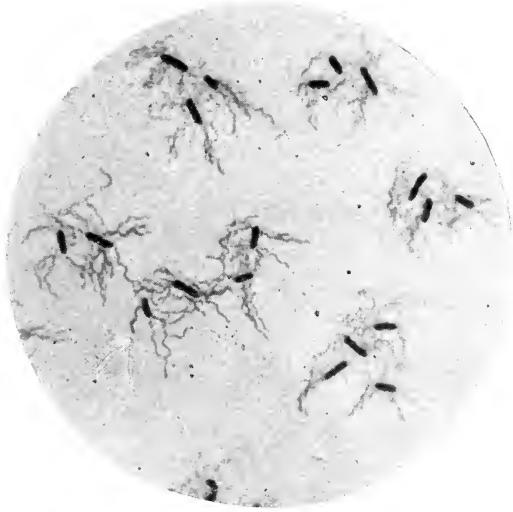


Plate 114. Bacillus Mesentericus Vulgatus, Flagellated

Photomicrograph of Bacillus Mesentericus Vulgatus: Isolated from slimy peas; cause of muddy liquor in canned peas. Bacilli showing numerous flagella, stained by Duckwall's method. Magnified 1,200 diameters.

all over the surface. It grows singly, often in pairs and short chains, and soon gives rise to spores which are quite resistant to heat. When cultivated on agar, the colonies are at first almost transparent, becoming bluish white and gradually extend, growing

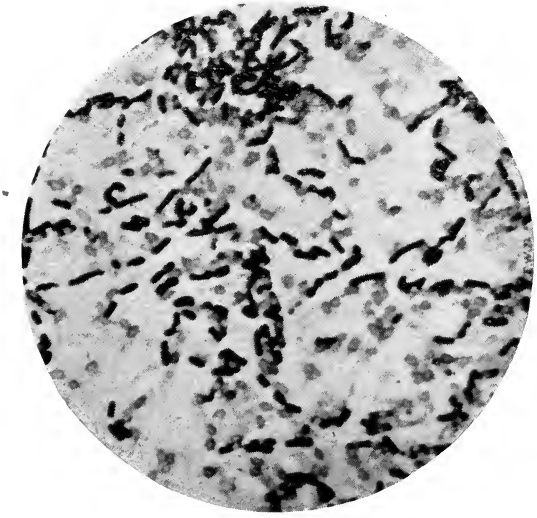


Plate 115

Photomicrograph of Bacillus Mesentericus Vulgatus, showing rods and spores. Taken from Agar culture and stained with fuchsine. Spores are very resistant to heat. Found in slimy peas. Magnified 1,500 diameters.

more opaque and wrinkled. The streak culture is a dirty white, and spreads rapidly over the whole surface, forming slime rapidly. On gelatin the growth is rapid, and liquefaction takes place along the entire line. It is aerobic and facultative anaerobic and grows well at room temperature, but most rapidly at 100 to 105° F., in which temperatures it forms spores very fast, and these are located near the center of the rods. After spores begin to form, the cells slowly dissolve into a slimy mass cementing the surrounding spores together. We have succeeded in photographing them thus as indicated in plate 46.

The slime produced by this organism is so mucinous that it may be drawn out into long threads by dipping the platinum loop into a growth on agar.

As may be imagined by our readers, it is quite difficult to obtain a slide preparation sufficiently free from slime to demonstrate by staining the flagella or organs of locomotion as shown in plate

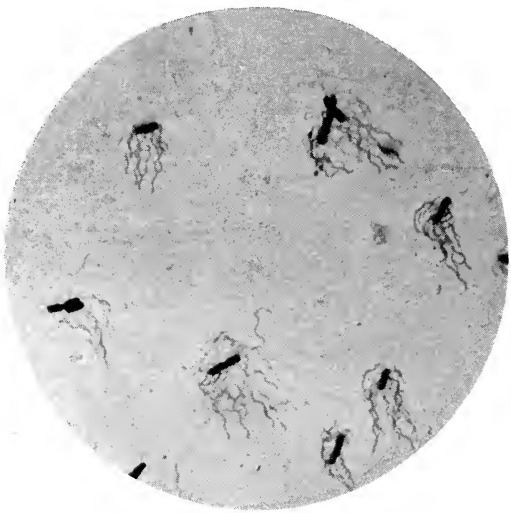


Plate 116. Butyric Acid Bacillus, Flagellated

Photomicrograph of a Butyric Acid Bacillus which softens cellulose. Isolated from peas during "sweating." Cause of souring and clouded liquor. Bacilli showing numerous flagella. Stained by Duckwall's method. Magnified 1,200 diameters.

114; but it may be done by inoculating a test tube of bouillon, and after a growth is obtained, the surface of agar is streaked with a small platinum loopful of the culture. Within six hours a thin, almost transparent film will be seen to be extending over the surface, and from the edge of this a growth of the bacilli sufficiently young and free from slime may be obtained for the demonstration of flagella.

When the mucinous matter forms on the peas, it cannot be entirely removed in the blanching bath, because the cellulose or fibre has been softened and the bacteria have gained entrance to the interior, so during the final processing this matter, together with the mealy substance of the peas, is diffused throughout the liquor which, of course, gives it a muddy appearance.

As we have explained in previous pages, the sweating processes either among the vines or shelled peas are the chief causes of souring, so also they are the chief causes of muddy liquor.

There is another bacillus which produces butyric acid, and is strictly anaerobic, which condition is produced in the center of heated vines and peas, the oxygen being all utilized by the bacteria on the surface of the mass. This organism resembles bacillus butyricus amylobacter, but seems to differ from it in spore formation. It is actively motile in the vegetative state, having numerous flagella.

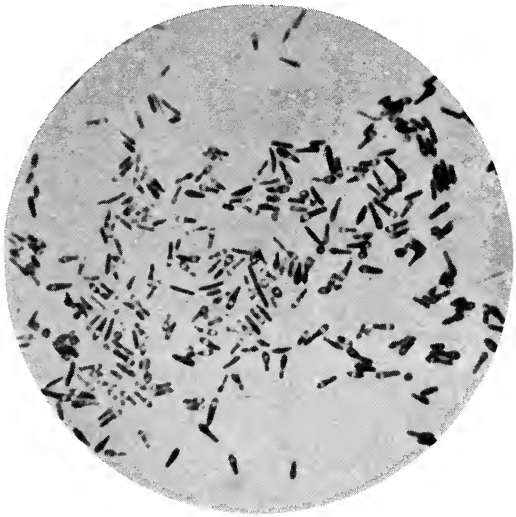


Plate 117

Photomicrograph of a Butyric Acid Bacillus which produces terminal spores. Isolated from peas during "sweating." Cultivated by Pyrogallate method, stained with fuchsine. Magnified 1,000 diameters.

I found this bacillus in the central portions of a basket of shelled peas which had stood over night. There was a perceptible odor of normal butyric acid, and the peas were quite hot and slimy. The organism did not grow in the Petri dishes, so I inoculated a small test tube, and tried the pyrogallate method, described in Chapter III, for the cultivation of anaerobic bacteria. The organism grew quite well, and is about 2 μ long and 9.7 μ in breadth, giving rise to terminal spores very much resembling Bacillus

Tetani, which is the cause of lockjaw, described in Chap. II. (See Plate 27.) The spores were not so near the end of the rods, however, some being near the center, but generally pretty close to the end. See Plate 48.

This organism rapidly softened the cellulose and the skins of the peas became quite soft. I used the utmost care in blanching, and filled the cans with filtered brine, but after the sterilizing process the liquor was very muddy and viscid. The inside of the peas was soft and became diffused throughout the contents of the cans, so I had pretty good evidence that the bacillus in question was the cause. Frankel and Pfeiffer isolated an organism very similar to this from a rotten melon, and Omelianski describes an anaerobic bacillus having terminal spores which softened cellulose, produced butyric acid, carbonic acid and hydrogen corresponding closely to this organism found in the peas.

There are other varieties seen in bacteriological examinations, which produce changes of this kind, set free volatile and fatty acids, and impart unpleasant flavors to peas which are allowed to stand exposed, so we call particular attention to the necessity of quick work between the cutting of the vines, and the sterilizing process.

Another cause of muddy liquor in cans of peas is due to overfilling the cans with peas. We should always bear in mind that peas swell some during sterilization, and if filled too closely will pack and this causes the skins to burst, permitting the mealy parts of the peas to become diffused throughout the contents. This is a feature of pea packing not generally known among canners, so I desire to call particular attention to the fact that the cans must not be filled too full of peas. This difficulty is obviated by the employment of modern filling machines and is not so common as it was in the days of hand filling. The measures in the machines should be set to hold just enough peas, so that the cans will be filled up to three-quarters of an inch from the top.

Overprocessing is another cause of muddy or cloudy liquor. If the peas are cooked to pieces, of course, the liquor will not be clear. To properly sterilize the peas without cooking them to pieces requires more judgment than any other step in the process of pea packing. There are so many sizes, all requiring different time, and the nature of the peas must also be taken into consideration—some varieties requiring longer time than others.

Another cause of clouded liquor is imperfect sterilization, and by that I mean that all bacteria are not destroyed. There are some bacteria which have spores of great resisting powers against heat, and if they are not destroyed will develop in the cans where the sterilizing process is insufficient. Swelled cans usually result, but there are some varieties of bacteria which do not produce gas

when growing in some substances and canned peas seem to favor this phenomenal characteristic.

As a rule these bacteria produce volatile and fatty acids which expand when the cans are heated, but at ordinary temperatures there is no outward indication that the contents have undergone chemical changes. Such cans of peas are sour, of course, and the liquor is clouded, because it is the culture medium for these bacteria. Sour peas, due to imperfect sterilization, are exceptional, because, as I have said, the cans more often swell; but I have seen a few cases.

When the sterilization process is not complete, it generally happens that swells result, but this is not always the case. There are a number of bacteria which may cause souring without the gen-

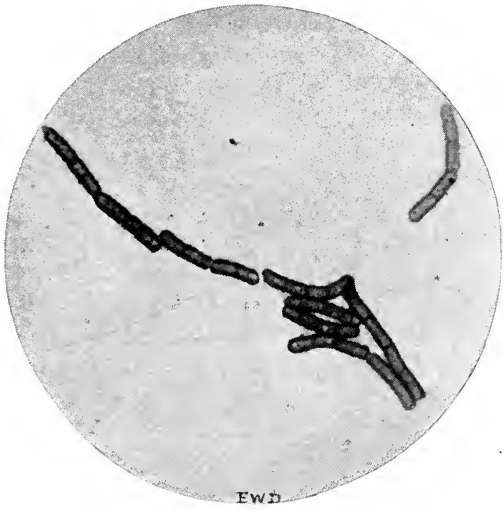


Plate 118. Bacillus Megatherium

Photomicrograph and Slide Preparation by the Author. Bacilli growing in chains, vegetating forms showing segmentation. Culture isolated from can of sour peas. Stained with tannic acid and gentian violet. Magnified 1,000 diameters.

eration of any gas. Some bacteria which produce gas when growing in some goods do not produce it when growing in other goods.

The anaerobic bacteria generally produce much gas, and the extremely foul odors present in swelled cans of peas are ordinarily due to them, while the souring is more frequently caused by germs which are aerobic and facultative anaerobic. Some of these, however, form sulphuretted hydrogen which has a very unpleasant odor.

When sterilization is incomplete the spores are not killed and after a short time they begin to vegetate, utilizing elements necessary for their growth—viz., carbon, oxygen, nitrogen, hydrogen,

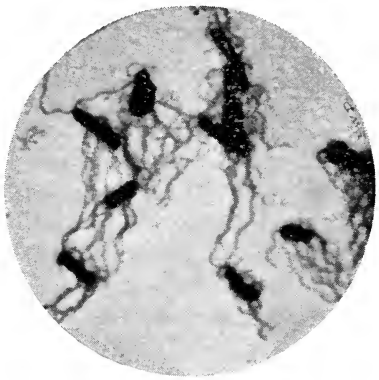


Plate 119. Bacillus Megatherium

Showing Flagella from Agar culture, eight hours' growth. Bacilli isolated from can of sour peas, showing much slime in the liquor. Photomicrograph and slide preparation by the author. Flagella stained by special method. Magnified 1,200 diameters.

etc., and these elements are obtained by breaking up the molecules containing them, causing marked chemical changes most noticeable in the sugar or carbohydrates. The sugar is broken up and there are formed various acids or alkalies according to the nature of the organisms at work.

Lactic acid is found by a large number of bacteria, and this is one of the chief products which gives peas a very sour taste. In

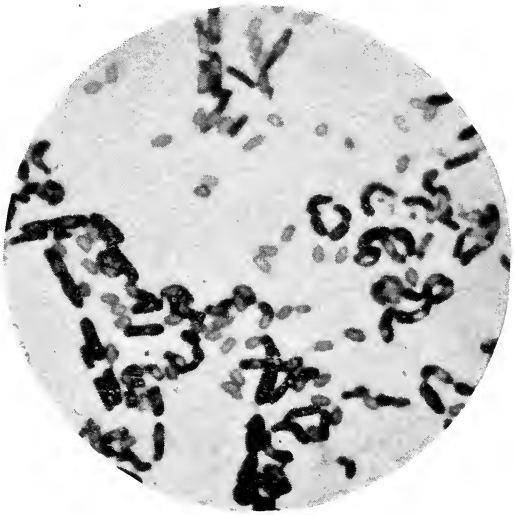


Plate 120. Bacillus Megatherium

Showing spores free and forming in the rods. Forty-eight-hour culture on Agar obtained by plate method from can of slimy sour peas. Photomicrograph and slide preparation by the author. Stained with fuchsine. Magnified 1,200 diameters.

nearly all cases of souring the liquor becomes quite muddy, often viscous. Slime is formed by several species belonging to the well-known aerobic spore-bearing bacteria. I have seen the liquor on canned peas so slimy that threads more than a yard long could be drawn out of the cans by dipping a platinum wire into the juice and slowly drawing it out. The organism at work in this case was Bacillus Megatherium.

This organism was first discovered by De Bary on boiled cabbage, but is quite common and widespread on various vegetables. It is a bacillus having round ends, quite large, being 5 to 6 μ long and about 2.5 μ thick. It forms chains of several members, and when stained in a certain way may show segments (see Plate 118) or division lines, so that a single bacillus will appear to be composed of three or four members, which is indeed the case, each one having the power to grow out into the long form so that a chain will result, looking something like sausages. When forming spores the cells are nearly filled (see plate 120) and the cell seems to dissolve away, leaving a somewhat smaller spore than we would expect to see. These spores are quite resistant to heat and are able to stand boiling for some time. The special character of megatherium is that it is slowly motile, although possessing numerous flagella, produces cloudiness and slime, forms abundant sulphuretted hydrogen, has no indol reaction and forms spores rapidly.

The peas which contained this organism had been processed for 25 minutes at 240° F. Many of the cans were swelled and in some of these I found the butyric acid bacteria described in previous pages. Another case of sour peas caused by insufficient sterilization came to my notice some time ago when the party had filled his retorts with water to a certain height and then attempted to process them with a steam supply at the top (instead of the bottom) of the retorts. Of course, all the peas below the surface of the water soured, and the most of them swelled. One peculiar feature of this case was a complete bleaching out of all the natural color of the peas; the chlorophyl had succumbed to the sulphur gas formed in the can. This gas had been generated by a certain species of anaerobic bacteria.

In the sour peas, of which there were quite a number, I isolated one which had produced considerable formic acid, also a slight per cent succinic acid. This organism was quite motile and did not form spores. It had numerous flagella and when grown on the surface of nutrient agar produced a beautiful red color. This was bacillus prodigiosus and under certain conditions grows wonderfully, sometimes giving off the odor of herring brine. It does not form spores, as we have said, consequently is easily destroyed. The process, therefore, was *very* ineffective where this can had

stood in the retort. The can must have been among those at the bottom. We have described this organism pretty fully under the head of chromogenic bacteria, chapter II.

One can which I examined showed a very clouded liquor, but was not so slimy. The peas in this can were quite firm and had a rather raw taste, due to insufficient cooking. An examination of the liquor showed the presence of very motile bacteria, some of which were extremely long. Even the longest of them had a serpentine motion, so I made inoculations in agar and put the dishes in the incubator for development of colonies.

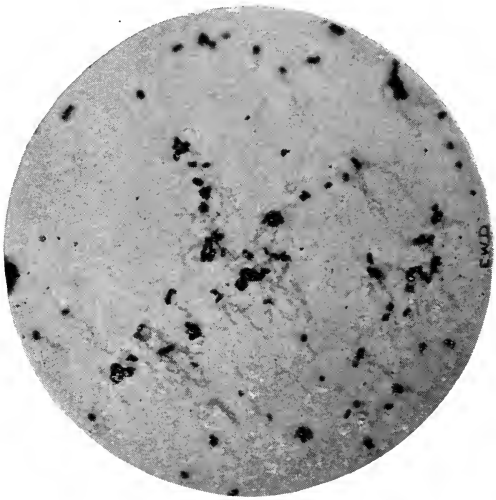


Plate 121. Bacillus Prodigiosus

Photomicrograph and slide preparation by the author. The staining was done by special method for the demonstration of flagella. This organism was isolated from a can of sour peas. On Agar produces a beautiful red color or pigment. The bacillus is sometimes called Monas Prodigiosus and "Bleading Bread," because it forms red spots on bread. Magnified 1,000 diameters.

On the following day small, irregular, shining white colonies made their appearance and grew rapidly, sending out little hair-like threads in all directions, visible when magnified about fifty times. A thin, almost transparent bluish film soon spread rapidly to the walls of the dish. From the edge of this film I obtained a specimen for staining flagella. The organism has all the characteristics of Bacillus Subtilis, or the Hay Bacillus.

After two days the culture began forming spores which are located near the center of the rods. These spores, when inoculated into good cans of peas, produced the same cloudiness seen in the original can from which the colonies were isolated. The spores are quite large and seem to have a very thick membranous wall, as shown in the plate. The walls of the spores take the stain well,

while the center of the spores remains uncolored. The dye is not able to penetrate to the center, although heated for some time directly over a flame until steam arose from the cover glass. This organism and those similar to it are among the great heat-resisting bacteria, requiring fully ten minutes' exposure to 250° F. before being devitalized.

The question is often asked, "How long shall I process my peas so they will keep?" This cannot be answered in a manner to cover all conditions and sizes, but we can give the results of quite a number of experiments to demonstrate the effect of certain temperatures on peas which were put through rapidly from the time the vines were cut until they were put into the retorts for sterilization. I made a number of experiments with peas, canned in the manner often seen in canning houses, where the vines were allowed to become heated. In the latter case the sterilization seemed to be more effective than peas put through quickly, but muddy liquor resulted in a number of cans, showing that the cellulose had become much softened. I attribute the more complete sterilization to the fact that all spores were probably giving rise to vegetating rods which are easily destroyed by boiling temperature. Many cans were sterilized completely in twelve minutes at 240°. The cans were placed in an incubator, where the temperature was maintained at blood heat, which is favorable for the growth of most bacteria. Out of twelve cans processed at the temperature given above only two swelled and the balance kept all right. I opened them after several months, and although the liquor was clouded, the peas were not undergoing decomposition; the bacteria were all destroyed. If any spores had been present before the sterilizing process the temperature and time of exposure would have been insufficient to destroy them. In order to demonstrate that all bacteria in these cans were dead after sterilization, I streaked a number of Petri dishes containing nutrient agar with the juice of the peas, then placed them in the incubator in a temperature of 98° F., where they remained until the agar dried up, without showing any signs of bacterial growth.

After this experiment I tried a number of cans of very small peas taken from the vines as quickly as possible, and covered them with good clear brine and then processed them for 12 minutes at 240° F., the same as I had given the cans in the previous experiment. In two days all of these cans swelled in the incubator and I removed them. The pressure from the gas generated within the cans was great and when punctured the juice squirted out with considerable force. An examination of this juice by the hanging drop method revealed a number of rod forms showing motility. There were several kinds, some of them quite active, spinning,

Bacillus Subtilis, Ehrenberg

HAY BACILLUS.

Origin.—In water, soil, faeces, putrid fluids and in infusions of hay.

Form.—Large, rather thick rods, having rounded ends and being three to four times as long as wide. Found usually in pairs; frequently in threads.

Motility.—Actively motile; snake-like motion; having from eight to twelve flagella.

Sporulation.—Large, oval spores are formed at or near the middle, without enlargement; these are highly resistant, and may be double stained readily. Germination.

Anilin Dyes.—Stain readily, as does Gram's method also.

Growth.—This is very rapid; cell division has been observed to take place in seventy-five minutes at 21° and in twenty minutes at 35°.

Gelatin Plates.—The surface colonies liquefy gelatin rapidly and extensively. The central portion of the colony presents the appearance of a grayish-yellow, irregular mass; on close examination it is seen to be made up of moving cells. This is surrounded by a lighter, granular zone. The border is quite characteristic, consisting of a dense zone of bacilli and threads, radially arranged, the ends projecting outward, presenting a striking appearance—the so-called "ray crown."

Stab Culture.—Funnel-shaped liquefaction takes place very rapidly along the entire line of inoculation. White, flocculent masses accumulate at the bottom, the liquid above, which is at first turbid, becoming clear. A dense white scum or zooglea is usually formed on the surface.

Streak Culture.—On agar, a dull grayish-white, thick, folded scum is formed. It develops well on potato, forming a moist, thick, yellowish white covering, at first velvety in appearance, but later becoming dry and granular, which contains spores as well as involution forms. On blood-serum it forms a folded scum and liquefies.

Oxygen Requirements.—Aerobic.

Temperature.—It will grow at from 10° to 45° C. Best at about 30° C.

Behavior to Gellatin.—Liquefies rapidly and extensively.

Pathogenesis.—It has no effect. If spores are injected into the blood they soon disappear, being taken up by the liver and spleen. They may preserve their vitality after being stored up in these organs for sixty to seventy days. (Wyssokowitsch.)

A large number of bacilli resemble to a marked extent the Bacillus subtilis. It is, therefore, customary to speak of the group of hay bacilli.

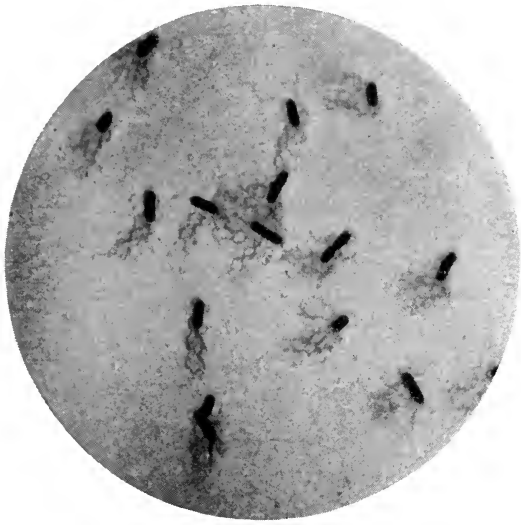


Plate 122. Bacillus Subtilis

Vegetating rods from a very young culture on Agar. Bacilli showing flagella. Magnified 1,000 diameters.

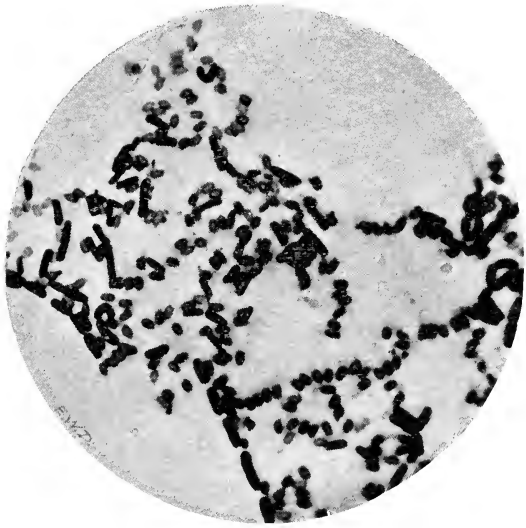


Plate 123. Bacillus Subtilis and Spores

Photomicrograph and slide preparation by the author. The spores have very thick cell membrane almost impenetrable by heat. This germ is also called the hay bacillus, having been found first in hay. This specimen was obtained from can of sour peas having cloudy liquor. Isolated by plate culture in Agar. Magnified 1,000 diameters.

turning over and over in somersaults; some were slower in motion, having a tendency to stand on end and to collect in bunches near the center of the drop of juice. These were the anaerobic species which do not thrive in the presence of oxygen.

The activity motile rods belonged to the aerobic species and are able to live also in an anaerobic condition. I isolated the various forms, some of which would not grow at all on the surface of the Petri dishes. The anaerobic species developed well in test tubes containing agar by making the inoculation with a platinum needle plunged clear to the bottom. These tubes were placed in the anaerobic culture apparatus, where all oxygen is replaced by hydrogen or absorbed by neutralized pyrogallic acid. (See Chapter III.)

I made some experiments with the self-registering thermometer to ascertain how long a time was required for the temperature in-

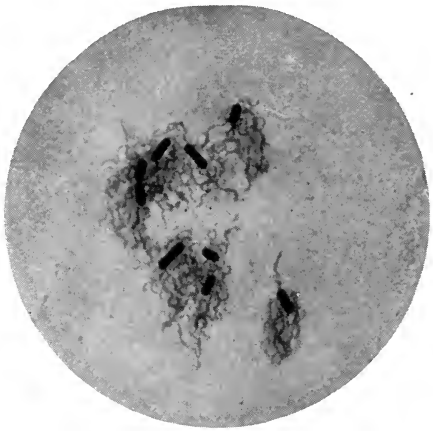


Plate 124. Anaerobic Bacillus from Peas

Vegetating rods from a culture grown by Pyrogallate method in a stab culture in Agar. Flagella very much curled and twisted, giving rapid motility to the cells. Staining done by author's special method. Photomicrograph with acetylene light by the author. Isolated from swelled can of very young peas. Magnification, 1,200 diameters.

dicated on the retort thermometer to register at the center of cans of peas. The self-registering thermometers are made in two ways. One is made to fit a can specially constructed for the purpose; it is screwed downward from the top and is sealed with a gasket, the mercury column being exactly in the center of the can. The other kind rests on a tripod, which may be inserted into the regular can and held in position by the legs of the stand. The first kind is not suitable from the fact that the gasket frequently leaks, and there is more metal used in constructing the special can, thus preventing the heat from penetrating to the center as rapidly as it does through the regular can. The other kind has the advantage because it is

sealed up within the regular can and is always under the same conditions as the goods which are being packed.

The mercury column in the self-registering thermometer is made with a constriction near the mercury bulb; so whenever the column rises to a certain mark it remains stationary until the can is opened. In this way the maximum temperature will be indicated.

To make a series of tests to find out the length of time required for certain temperatures to reach the center of a can, you will proceed in the regular manner by gradually raising the temper-

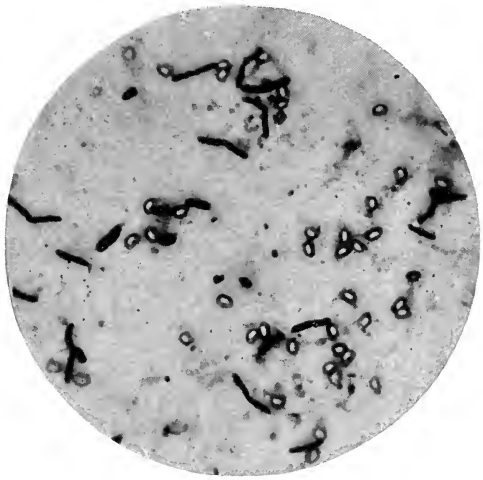


Plate 125. Anaerobic Bacillus X.

Showing rods and spores. Formation of spores was rapid at 98 degrees F. in incubator. This bacillus produced great quantities of gas. Isolated from swelled can of very young peas. Odor from culture very foul. Pure culture obtained by the pyrogallate method. Photomicrograph with acetylene light by the author. Magnified 1,200 diameters.

ature to 230° F. for first experiment. After maintaining this degree of heat for twenty minutes you allow the pressure to decrease, chill the can, open and examine the thermometer, and you will find that it indicates only 225° F. At 240° F. for twenty minutes, 230° F. is registered at the center. At 250° for twenty minutes only 235° have been registered.

After a number of tests I have prepared a table which shows the time required for the heat to register at the center of the cans. using different lengths of time and different degrees of heat.

I made a number of experiments to determine the length of time required for a given temperature to register at the center of a

can of peas. I found that the time required was much less than that for corn, owing, no doubt, to the more fluid nature of the contents. The juice of the peas makes a very good carrier of heat from the tin to the center of the cans. There is no doubt that the center of hard peas is less penetrable, consequently such peas will not have the recorded degree of heat inside, even though the liquor does register a certain temperature. There would be only a small difference with small size peas, but the older varieties would probably need a few minutes extra time. To show the result of experiments with self-registering thermometers I will give the results as recorded in my notebook. The left hand column will show the number of minutes given, while the different degrees, as indicated by the retort thermometer, are at the heads of the columns:

TABLE SHOWS HEAT PENETRABILITY OF CANS OF PEAS.

Minutes	230	235	240	245	250
20	225	227	230	232.5	235
25	226	229	232.5	236	240
30	227.5	231	235	239.5	244
35	229	233	237.5	242	248
40	230	235	240	245	250

A careful study of this table brings out some important facts: 230° F. for forty minutes is the same as 240° F. for twenty minutes; 235° F. for forty minutes is the same as 240°F. for thirty minutes and 250° for twenty minutes. It requires about forty minutes for a given retort temperature to be recorded at the center. Peas processed at 250° for thirty minutes reach 244° F., which makes an average of 237° F. for at least fifteen minutes.

I made a number of experiments, using various temperatures, for sterilizing peas. I boiled twelve cans in open bath for two hours, then put them in the incubator to favor the development of bacteria, should any be left alive inside the cans. Five spoiled within one day, three spoiled by the end of the second day, and two more swelled the following day. Two cans seemed to be all right. They did not swell, although kept in the incubator at blood temperature for three weeks. On opening these cans, however, they were exceedingly sour. The color of the peas was much bleached, but otherwise they looked fairly well, except that the acid was strong and surprised me very much when I tasted them.

I melted a flask of agar in the autoclay at 240 degrees F., then filled six Petri dishes. When cooled to about 120 degrees F. I inoculated two with several loops full of the liquor from these cans, then made transfers from the first two into two more, and from these made transfers into two more. I placed the dishes in the in-

cubator and at the end of twenty-four hours, the two first dishes were completely covered with a thin growth extending even up to the walls.

The next two dishes had a few colonies, but the greater portion of the surfaces were covered with a spreading growth. The two last dishes contained a few scattered colonies which formed in wedge shape or whetstone shape below the surface, and grew upward, forming thin, round colonies on the surface. Some of these colonies had a brown, opaque, granular appearance by transmitted light, but on reaching the surface had a bluish cast with a shining

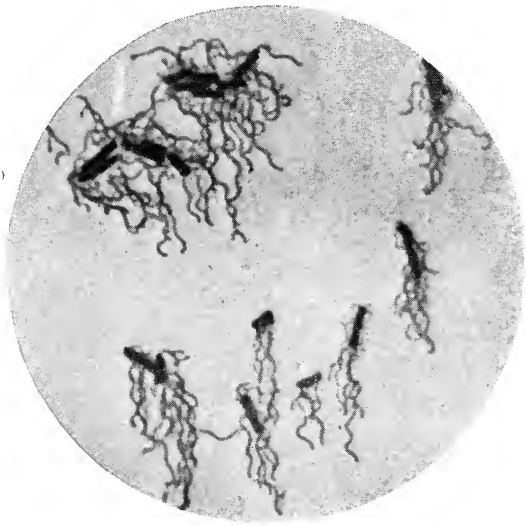


Plate 126

Photomicrograph of Bacillus X found in can of sour peas. Forms no gas and reduces sugar to acids. Bacilli having numerous flagella, which are stained by author's special method. Magnified 1,500 diameters.

transparent luster. The surface growth extended gradually to about the size of a silver three-cent piece, the lustrous appearance giving way to a dull scum-like layer quite viscous and forming folds or wrinkles, becoming much elevated.

When transplanted to gelatin the colonies began to sink into the gelatin, in thirty-six hours leaving saucer-like depressions, and a floating film formed on the surface of the liquefied gelatin. The growth in bouillon was rapid, producing very little cloudines, a pellicle forming on the top which grew fast to the wall of the test tube.

Colonies on agar, when viewed by carefully lowering a 1/8 dry objective into focus, present a wonderful appearance. The bacilli are seen in active motion, travelling in parallel lines, in curves, ex-

hibiting their motility in twisted, writhing masses. By making a suspension in distilled water and carefully staining, I was able to demonstrate the flagella or organs of locomotion.

The cultures all had the odor of sulphuretted hydrogen, and in a few days the sulphur would be strong enough to bleach out the blue color made with the marking pencil on the glass Petri dishes and test tubes. I inoculated several cans of peas which had been sterilized, with some of these organisms and in two or three days the cans had the same appearance and taste as the original two cans. I tested other cans by inoculating them with spores from this organism and processing at 240 degrees F. for twenty-five minutes. Of the twelve cans so treated only two were sterilized. Ten of

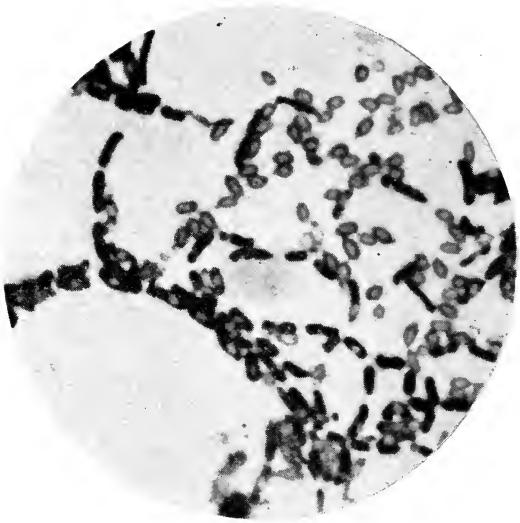


Plate 127

Photomicrograph of Bacillus X showing rods and spores. Spores are free and located in the cells. Spore Membrane is quite thick, giving them great heat-resisting power. Magnified 1,500 diameters.

these cans had a natural appearance, showed no signs of swelling, but turned sour in five days in the incubator. The spores of this organism are quite large, are situated near the middle or perhaps a little nearer the end of the cell. The cell seems to split open at the side, thus setting the spore free.

One dozen cans sterilized for twenty-five minutes at 250° F. kept all right, but it was no doubt very close to the danger line.

I tried one dozen cans heated to 220° F. for forty minutes, but all spoiled; some swelled and burst the cans, others simply soured and the liquor became much clouded. An experiment with six cans at 230° for forty minutes gave pretty good results, only

one of these spoiled, so I began an investigation of the causes of the spoilage. I made a series of inoculations in Petri dishes as previously described, and in twenty-four hours had a very fine growth of a germ answering every published characteristic of bacillus mesentericus ruber, commonly found on potatoes.

This is a somewhat slender bacillus discovered by Globig, who isolated it from boiled potato. It is a chromogenic bacillus forming a pinkish yellow pigment. It is an aerobic organism, but is able to grow fairly well where oxygen is almost excluded. I remem-

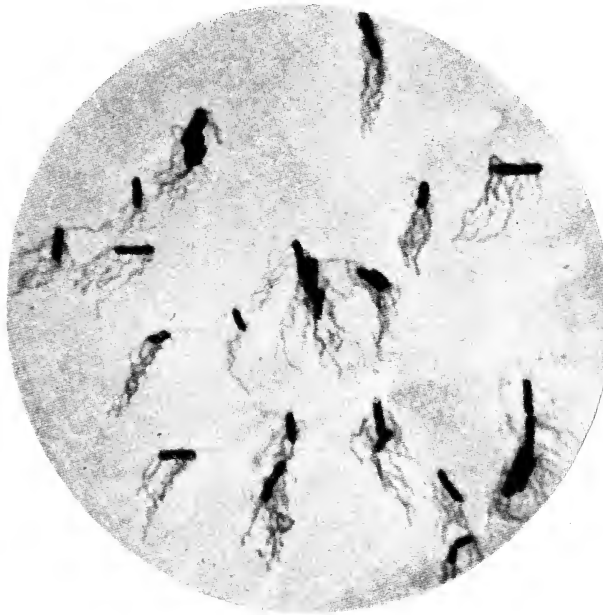


Plate 128

Photomicrograph of Globig's Bacillus Mesentericus Ruber, a chromogenic actively motile, Bacillus having numerous flagella, as here shown. This Bacillus was isolated from a can of spoiled peas and stained by author's special method. Culture from Agar eight hours' growth. Magnified 1,000 diameters.

bered well that the can I had opened was not full by nearly one inch, so that enough oxygen was present to give sufficient of that element for the growth of the bacilli. The colonies on agar were a yellow color, and those which lay just under the surface came rapidly to the top and began spreading. Under a magnification of fifty diameters, fine points were visible, extending outward from the veil-like growth. The growth on gelatin is similar, except that liquefaction begins as soon as the colonies begin to spread. On the surface of the liquefied gelatin a pink-like film forms. In the test tube stab culture this film spreads to the walls of the tube. The streak on agar is viscid with a transparent growth extending always in

advance of the filmed and wrinkled layer, and from this thin growth we are enabled to get the most actively motile rods. The flagella of these rods are always very numerous, and enable them to travel forward, rapidly spreading over the whole surface. The spores of this species are probably more resistant than any of the very common varieties of bacilli, excepting those of bacillus subtilis, and some varieties common to the soil. They are larger in diameter than the cell itself and are oval. The spore wall is quite thick, which accounts for its great resistance to heat.

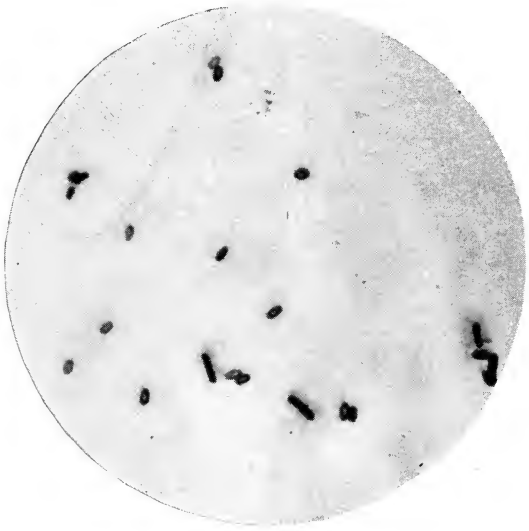


Plate 129

Photomicrograph of rods and spores of Globig's Bacillus Mesentericus Ruber. Spores are larger than the rods in breadth. Spores are able to withstand boiling for six hours. This is a chromogenic actively motile, aerobic organism first found on potatoes. It is widespread, being found on peas, corn, beans, etc. Magnified 1,000 diameters.

When these spores are inoculated into cans of peas they soon spoil and it is remarkable that only one of the six cans processed at 240° F. contained any of this species. I believe that if the cans are well filled with liquor the small amount of oxygen present would likely interfere with the growth of this bacillus. Cans filled nearly full and exhausted contain little or no oxygen, and all bacteria would soon be forced to grow in an anaerobic condition.

A certain packer once sent me a few cans of peas which he had processed at 240° for twenty-five minutes. Almost half were sour, having living bacteria present in them. The liquor was quite cloudy and when viewed under the microscope, in a hanging drop, motile organisms were seen moving quite freely, sometimes singly, but generally two together. They were slender and were endowed

Plate 130

Photomicrograph of Bacillus W, an actively motile aerobic and facultative anaerobic Bacillus found growing side by side with an anaerobic Bacillus in can of spoiled peas. The numerous flagella are demonstrated by author's special method. Agar culture eight hours' growth. Magnified 1,200 diameters.

Plate 131

Photomicrograph showing rods and spores of Bacillus W. Found growing with Bacillus K in peas which had been processed at 240 degrees F. for twenty-five minutes. From Agar culture four days old. Stained with fuchsine. Magnified 1,000 diameters.

with numerous flagella. I isolated these by plate culture and found that two species were present, one belonged to the group of anaerobes and refused to grow on agar, except in an atmosphere of hydrogen or by the pyrogallate method. I was able to get a good growth of both by a stab culture in agar of one, and a surface growth of the other variety. These two organisms had been accustomed to grow in the same goods, one being an aerobe, utilized all the oxygen obtainable and produced a condition of anaerobsis which was favorable for the growth of the anaerobic species. The other bacillus, although an aerobic, was also a facultative anaerobe, consequently flourished well in either condition. This phenomenon is often seen in bacteriological examinations, in so much that we have difficulty at times in separating certain species.

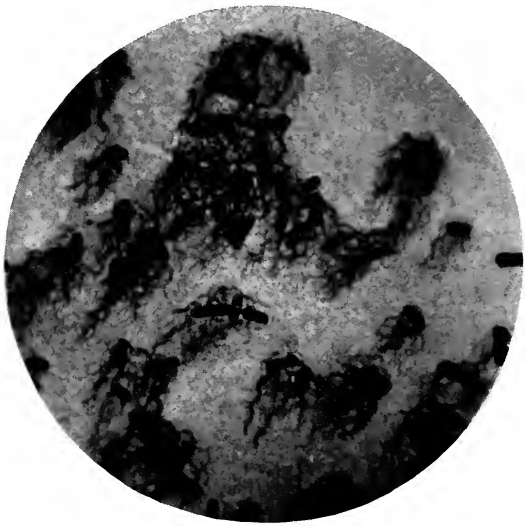


Plate 132

Photomicrograph showing Bacillus K. An actively motile anaerobic Bacillus found growing together with Bacillus W in can of spoiled peas. This was isolated by the pyrogallate method. Very numerous flagella. Magnified 1,500 diameters.

Both of these species began to form spores in three days, and I inoculated several cans of peas with them and gave them 240° F. for twenty-five minutes, and then put them in the incubator at 98° F. About one week later I opened these cans and they were all right. This seemed strange, because the packer who had experienced the difficulty had given them this very process, so for a time I was puzzled to know why my experiment had failed to show signs of spoilage. I streaked agar plates with the juice of my cans, but did not obtain any growth of bacteria. The only explanation I am able to make is that his peas were larger and harder

than those in the cans which I had inoculated. I had noticed this when I opened the original cans.

There is, of course, another explanation; his thermometers and gauges might have been incorrect, but the first explanation is probably right, because a process of 240° for twenty-five minutes is too short for peas, excepting perhaps the very smallest sizes. Even the small sizes may not be perfectly sterilized at this temperature. From our table showing the penetrability of heat by the test of inside thermometers, we find that only 230° F. is registered at the center of the cans in twenty-five minutes at 240 degrees, and this

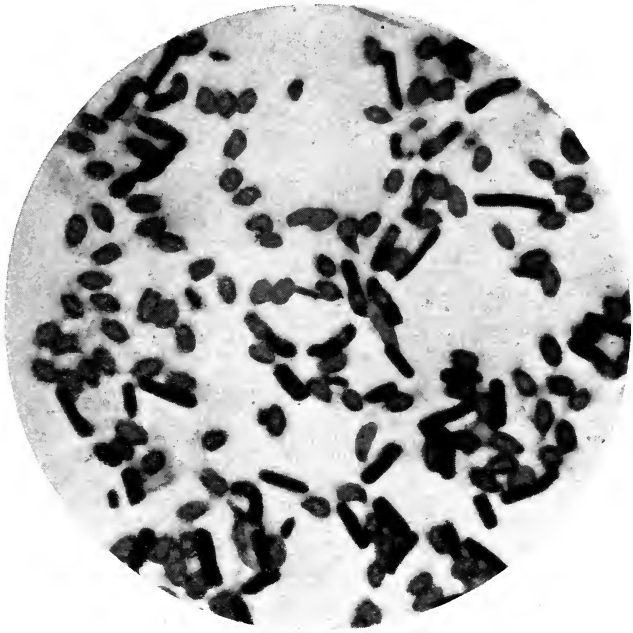


Plate 133

Photomicrograph of Bacillus K showing rods and very small spores. Bacillus K is an anaerobic organism found growing with the aerobic Bacillus W. in a can of peas which had been processed at 240 degrees F. for twenty-five minutes. Slide prepared from a four days' growth on Agar at 98 degrees F. in incubator. Stained with fuchsine. Magnified 2,000 diameters.

is hardly sufficient to destroy the spores in all cases.

We must not lose sight of another very important factor in dealing with spores. Old spores, those which have become dried or have been in a resting state for a long time, will be more difficult to destroy than the spores which have formed in laboratory cultures, which may be only several days old.

Laboratory cultures of bacteria, as a rule, are more readily destroyed by heat than the same bacteria which find a suitable substratum in peas in the field. The spores which get into canned

peas may be old; they may have been in a dormant state for a year or more, and consequently are dried, and the spore membrane may have shrunken and become more impenetrable. Our laboratory cultures of the spore-bearing species, usually form spores within a few days, and these, when transplanted into sterile cans of peas for experimental sterilization, are more susceptible to heat than the kind we have described. Our table of temperatures employed for sterilizing peas is best prepared from the results obtained in actual canning. A variety of spoilage cases are recorded in our notebook, and we will put the results of the various processes in table form, and then draw some practical conclusion, keeping in mind always, the various sizes of peas, also the location of the canning establishment:

Location	Size of Peas	Temperature (Degrees)	Minutes	Living Bacteria	Results
Canada	Marrowfats	240	30	present	About 20% sour, few swells
Canada	Early June	240	25	present	About 5% sour, cloudy liquor
Canada	Extra Early	240	25	present	About 5% sour, cloudy liquor
Canada	Petit Pois	240	25	present	About 2¢ sour, cloudy liquor
Canada	Petit Pois	238	30	none	Kept well
1. Mich.	Marrowfats	240	30	none	Kept, cloudy liquor } due to
Mich.	Extra Early	240	28	none	Kept } sweating
Mich.	Extra Small	240	25	none	Kept } of vines
2. Mich.	No. 4	240	30	none	Sour before canning
Mich.	No. 3	240	30	none	Sour before canning
Wisconsin	No. 4	235	35	present	10¢ sour, some swells
Wisconsin	No. 3	235	35	present	10¢ sour, some swells
Wisconsin	No. 2	235	30	present	10¢ sour, some swells
Wisconsin	No. 1	235	30	present	A few sour
New York	Marrowfats	240	35	none	Kept
New York	Extra Early	240	30	present	A few sour and swelled
New York	Small	240	25	present	A few sour and swelled
Ohio	Marrowfats	240	40	none	All kept
Ohio	Early June	240	35	present	A few sour and swells
Ohio	Extra Early	240	30	present	A few sour
Ohio	Small	240	25	none	All kept
Indiana	No. 4	240	38	present	A few sour, some swells
" branch factory	No. 4	235	38	none	All kept
Indiana	No. 3	235	38	none	All kept
Indiana	No. 2	235	35	present	Sour and cloudy liquor
Indiana	Petit Pois	235	30	none	Cloudy liquor { sweating of hulled peas
2. Indiana	Early June	240	35	present	{ Large number swelled Exhaust line clogged
Illinois	No. 4	240	35	present	A few sour
Iowa	{ Med. size Ex. Early }	240	40	present	A few sour, cloudy liquor
Penna	Telephone	250	25	present	Soured after 2 weeks
Penna	Telephone	250	30	none	Kept well
Penna	Early June	250	30	none	Kept well
Penna	Very Small	250	25	none	Kept well

After studying these different cases we see that the chances of spoilage at 240° F. for any time less than forty minutes is great, and we are impressed with the conviction that 240° F. is not a very reliable temperature. Perhaps in Canada and the northern states 240° F. for forty minutes is sufficient for all large and medium sizes and the very small sizes might keep well at the same temperature for thirty-five minutes, unless the weather should be hot and rainy, in which case the temperature should be increased.

In the central states we should recommend 250° F. for thirty

minutes for large sizes, and twenty-five minutes for the small sizes.

This cannot be laid down as a positive rule, however, but we may outline a definite method of ascertaining positively the best temperature under all conditions.

If the peas are worked up promptly and not allowed to go through any sweating process, the skins being firm and strong, we would recommend that 250° for thirty minutes be tried first, and if they hold up all right and do not crack open and cloud the liquor, that process will give good satisfaction. The alum in the blanching bath will help to give firmness to peas, so that this temperature may be used with safety. If, however, the peas are too tender, the time should be cut down to twenty-five minutes and the result carefully noted. It is a good plan to have an incubator in some part of the factory, and a good microscope is almost indispensable. After a certain process, a few cans out of each batch may be placed in the incubator, and kept in a temperature of 98° F.—blood temperature —and if the sterilization is imperfect, there will be a growth of bacteria within twenty-four to thirty-six hours. Here is where a good microscope with a 1-12 homogeneous oil immersion objective, is most valuable. The cans in the incubator are removed one at a time, the juice poured out into a clean dish and drops of the juice examined carefully. A simple method of examination is made by placing a drop in the center of an ordinary microscope slide, then dropping a clean coverglass over this, so as to have the juice between the two pieces of glass. A tiny drop of cedar oil is then placed on the center of the coverglass, and the iris diaphragm is closed so that the hole for admission of light is about as large as a pin-head in diameter. The light is then focused with the concave mirror and the Abbe condenser; the 1-12 oil immersion objective is carefully lowered, by means of the coarse adjustment, until it touches the oil, and then it is brought into focus by the fine adjustment, being very careful not to let it crush through the glass, which might destroy the delicate lens. In this connection we might refer our readers back to the description of a hanging drop examination in Chapter III.

If the microscope is equipped with a mechanical stage, the fields of view may be changed with precision, and the presence of any motile bacteria may be noted very readily. By reference to the numerous plates of various species of bacteria found in peas, it may be possible that some will be found which bear close resemblance to our illustrations. If no bacteria can be seen by the methods described, it is a simple matter to stain a few coverglasses evenly spread with the juice. The staining methods described in Chapter III will help any one to accomplish this feature of the bacteriological technique with ease and certainty. It is not always

easy for beginners to find living bacteria, because they are almost colorless, and the fluid is apt to form currents and confuse any one not familiar with this kind of examinations. A little practice with stains first, and living bacteria afterwards, will soon help the beginner to get accurate results. After acquiring a little experience, the juice from a number of cans may be examined in a short time, and the presence or absence of living bacteria may be ascertained with accuracy. By following up the practical work with the scientific it becomes an easy matter to keep well informed on the safety of of every day's work. After a time the various results following each amount of sterilization, ordinarily given peas, will furnish to the trained eye—by the aid of the microscope and the appearance of the goods—all the necessary evidences of imperfect sterilization, whether too short or too much prolonged.

We can thus sum up the points brought out under the head of peas:

There must be no delays after the vines are cut. Good judgment must be exercised in directing the cutting to avoid overcrowding the viners. If the vines are piled up in cribs or stacks, lactic fermentation get well started even in a short time. During rainy weather the work must be close up; the wet vines will sour very quickly. When lactic acid forms in peas it is never neutralized afterward.

"Sweating" softens the fiber or cellulose, and such peas will cloud the liquor. "Sweating" to the extent of forming butyric, lactic and fatty acids, will cause "slimy" peas.

The cleaning, separating and hand picking must follow the viners quickly, to avoid souring.

The blanching should be done in running water if possible; a final cold water alum bath might be well recommended, then a spray of cold, clear water.

The filling should be uniform. Do not fill cans too full, about three-quarters of an inch from the top of cans is about correct.

For making brine use either spring, well or filtered water.

Never use saccharin as a sweetener, it is a violation of pure food laws in many states, and deceives the consumer.

Note.—The peculiar sweet taste of saccharine will soon prejudice the consumer against peas. I have made several experiment lately to ascertain this truth. Saccharin is not a food; it may have medicinal value in some cases, but when added to peas or other foods it is deemed an adulterant, and by various authorities is branded as injurious.

Use granulated sugar if a sweetener is necessary. Have good machinery and sufficient to avoid overcrowding at any time.

For sterilizing use 250° F., in preference to 240° F.; it is more reliable and saves time. Thirty minutes for large sizes and twenty-five minutes for small sizes may be stated as a broad rule—this, of course, may be varied if conditions make it necessary.

Always chill the cans before opening the retorts, by turning cold water into them. See diagram of process kettle, Chapter VI, Fig. 31.

The calcium system offers some advantages in sterilization. The revolving crates will permit the heat to penetrate to the center of the cans more rapidly than in the retort system. The time may therefore be shortened a little.

LABORATORY WORK ON PEAS.

SPOILAGE OF PEAS.

Some of the processes as given out from various sources are not altogether reliable, and such losses as have been reported up to this time are caused by insufficient sterilization. Insufficient sterilization is shown in two ways—the losses from swells and souring without any formation of gas. In the case of swells large quantities of gas are formed; this is due to bacteria belonging largely to the putrefactory organisms, especially to the anaerobic species universally distributed in the soil, the spores of which are found on the pods, leaves and vines of peas, carried there no doubt in particles of dust. The spores of anaerobic bacteria are not as resistant to the influence of high temperatures, as the spores of other varieties which do not form any gas. Some of the aerobic bacteria do not form gas when growing in cans of peas, and nearly all of the common spore bearing aerobes are able also to grow anaerobically. This class of bacteria causes the greatest and most complete losses in peas, corn, beans, asparagus, etc., because the decomposition is not evidenced by any swelling of the cans, and usually is not discovered until after quite a lapse of time. Sometimes the souring is quite slow, and does not show until two or three weeks after the peas are packed; sometimes the time is much longer.

Any sterilizing process which is followed by swells is very far below what it should be because, as we have said, that form of spoilage is accomplished by a class of bacteria less resistant to heat than the class which produces acidity without the formation of gas. In Bulletin 249 of the New York Agricultural Experiment Station at Geneva, Messrs. Harding and Nicholson made a number of experiments to determine the cause of the malodorous decomposition of canned peas, and also the amount of heat necessary to destroy the bacteria. The bacteria responsible for swells as they found them are anaerobes, and not the most resistant forms.

We have often isolated these from both peas and corn. They are almost as difficult to cultivate (on artificial media), as the Bacillus tetanus, or lockjaw germ. In our laboratory work we find that such organisms thrive best on a medium prepared with the juice of the very goods in which we find them growing. Frequently we find that we get a very poor growth on agar prepared with meat juice and peptone. After obtaining sub cultures, however, they do pretty well. The cultivation of anaerobic bacteria to obtain a superficial growth is quite difficult, and since this is the only kind of a culture that is fit for staining the young rods in order to demonstrate the flagella, or organs of locomotion, great care must be exercised in the technique. As we have stated, the anae-

Plate 134

Photomicrograph of a Bacillus found in can of swelled peas. This organism answers to the description of Bacillus Butyricus (by Piazmowski). This plate shows the motile vegetating rods. The germs were cultivated anaerobically by the pyrogallate method. Stained by our special method and photographed through 1-12 Spencer objective. Magnified 1,500 diameters.

robic bacteria grow only where oxygen is entirely excluded, that gas in the free state being poisonous to them, it must either be replaced by hydrogen or absorbed by chemicals. We are very successful in growing these anaerobes by the pyrogallate method, which is done by sealing loosely stoppered tubes containing the inoculated or streaked agar, in a jar, previously adding enough alkaline fluid to neutralize a given quantity of pyrogallic acid. This mixture is poured into the jar and the culture tubes are placed inside and the jar is then sealed absolutely tight with sealing wax.

We obtain the best results by first growing a pure culture in the clear juice, in the anaerobic jar, and then from this we streak

slanted agar tubes, using a liberal quantity of the juice to cover the surface of the slant. After two days we generally get a good superficial or surface growth. A small quantity of this growth mixed with water and thinly spread over the surface of a coverglass is generally all right for staining. The staining for the demonstration of flagella, as shown in Plate 134, is a very difficult task, requiring experience and judgment to obtain good results. Many failures may be expected ere a first-class preparation fit for photomicrographing is obtained.

Spore bearing bacilli of this class form spores rapidly, especially in the incubator, and when this stage is reached the flagella cannot be easily demonstrated. Many of those delicate hair-like organs of locomotion drop off and are dissolved in the surrounding fluid, leaving the rod with its bright spore nearly devoid of flagella. In such preparations the spores are seen both in the rods and also free, as shown in Plate 135. The spores are the resistant forms of bacterial life. They may be regarded as the seed forms. The spores are formed within the rods and the first indication of spore formation is the appearance of granules throughout the cell, which soon collect in a certain place, at first as an irregular mass, gradually assuming a round or ellipsoidal form and becoming brighter and more refractive, having a wall or distinct membrane. This membrane becomes thicker and protects the life within, just as the membrane, which surrounds a dry mustard seed, be an or pea. It prevents heat from penetrating to the inside; even boiling temperatures are resisted for several hours. Spores form in the rods only when conditions become unfavorable for multiplication. Reproduction or multiplication is the natural characteristic of bacteria, but this soon ceases in culture media, because the supply of nourishment is limited, and there is no provision for carrying off the products excreted by the bacteria. In cleavage processes the compounds which are formed are often poisonous to the germs and act as antiseptics. It is due to this fact that the antitoxins used in diseases are so valuable. The toxins are poisonous to the germs themselves, and render the medium upon which they are growing unfit for their multiplication, hence spores are formed which have the power to resist the poisons, until such time as they may find lodgment in a more suitable substratum. This power to resist an unfavorable environment is the secret of the difficulty experienced in sterilizing canned goods without injuring the quality. As a rule the line of safety in sterilization is so close to the line of danger from scorching that it is sometimes quite difficult to produce goods which will keep well and still retain much of the natural flavor. Sterilization will always change the flavor to some extent, but goods must not have a scorched taste or odor. It cannot be laid

down as a positive rule that peas will be processed properly at a temperature of 240° F. for 25 to 30 minutes. We have on record a number of spoilage cases where this temperature failed. While a number of packers will testify that this has always been sufficient for their peas, there are still others who have lost considerable money on account of sour peas. The only possible way to know just what temperature is sufficient is by bacteriological examination. I have sterilized peas perfectly at 250° F. for 15 minutes, but I know that it would be ruinous to use such a process universally. In the laboratory we have sterilized peas and corn, too, in 20 minutes' exposure to 240° F., but it happened that the raw product was free from some of the resistant varieties. It is pretty safe to start out with a process of 250° F. for about 25 minutes;

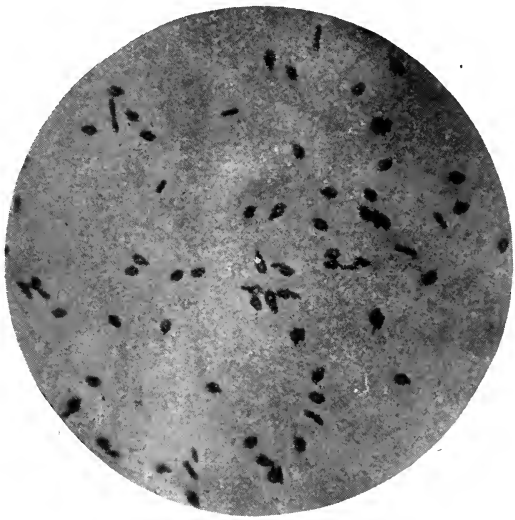


Plate 135

Photomicrograph of the spore forms of the Bacilli shown in Plate 134. The spores are terminals and much larger in breadth than the rods. These spores are resistant to high temperatures and vegetate only in an anaerobic condition. Photographed through the microscope under a magnification of 1,500 diameters.

some of the cans may then be placed in a warm place for two or three days, and the juice may then be examined under a one-twelfth homogeneous oil immersion lens. Since nearly all of the bacteria identified with spoilage are motile, the rods may be seen swimming around in the juice. We would recommend a temperature of 250° F. for 25 to 30 minutes. One or two cans sent to the laboratory by express will reach us within 24 hours in most cases, and we are well equipped to examine any goods and report by wire if necessary to increase the time.

The following letter is one which must be interesting to every packer because it is a description of the difficulties and losses which have befallen many, and seemed so mysterious, too:

Prof. E. W. Duckwall, care of National Canners' Laboratory, Aspinwall, Pa.

DEAR SIR:—We are sending you by express today, charges prepaid, a box containing six cans of peas. These were absolutely fresh stock, shelled, cleaned, filled and processed without any delay, and cooked under ten-pound pressure twenty-five minutes after the glass showed 240°; then cooled and stacked in cases. The swelling shows about a week after being cooked, and seems to be very general, from present appearance, perhaps one-fourth of the pack. We have, on noticing this condition, increased our cook to fifteen-pound pressure, or 250°, and find that we have the difficulty stopped, but we are at a loss to understand the conditions that existed in the first two weeks' pack. We have cooked our peas for the past three years at the ten-pound pressure, and from twenty to thirty minutes, and have had no trouble of any moment. This was, too, when our factory was crowded, and the peas did not go through the process as promptly as they have this season. Also, when they had to be delayed in threshing other seasons on account of excessive deliveries; in fact all the conditions in our factory this year are 25 per cent better than they have been.

Another point is, that we have insisted on our goods being delivered at an early stage of growth, and the result being that we have packed very much larger of high-grade goods and this loss coming at this time is exceedingly trying, and we would appreciate very much your assistance.

You may perhaps remember the writer meeting you at Columbus last February and talking over this very point of processing peas, and you expressed your views at that time that we would probably have to increase our temperature.

Thanking you for your interest in the matter, we beg to remain.

Very truly yours,

_____.

The six cans arrived, three of them bursted and contents gone. The odor was very bad, showing that putrefactive bacteria had accomplished the spoilage. The three remaining cans were badly swelled, and when punctured the force of the gas caused the juice to spurt out in a stream. We quickly streaked a number of petri dishes and agar slants, also inoculated some tubes of liquid culture media. Part of these were placed in anaerobic jars, so that the anaerobic bacteria might form colonies along with the facultative

aerobes. After two days quite a number of colonies made their appearance, some of each species. The anaerobic bacteria were rods which formed terminal spores as shown in Plates 134 and 135. The facultative anaerobic bacteria formed small colonies in the jar, but in the petri dishes the colonies developed better, where free oxygen was accessible. The colonies were found with a lustrous appearance, bluish white, with opaque centers. Under a magnification of 60 they appeared granular with rough margins. The agar streak was a dirty white growth in folds. Streaked from a fresh bouillon culture the growth was moist and spreading, and from this we were able to get a preparation for staining flagella, or organs of locomotion, as shown in Plate 136.

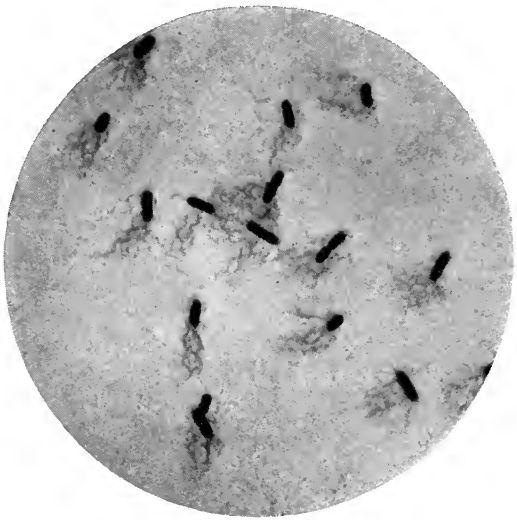


Plate 136

Photomicrograph of the pea bacillus, which resembles Bacillus Subtilis. This shows the young vegetating rods and the organs of locomotion called flagella. This organism produces spores of great vitality. It is difficult to destroy, much more so than the bacillus shown in Plates 134 and 135. Stained by author's method and photographed through the microscope. Magnified 1,500 diameters.

The spore formation took place in the center of the rod, as shown in Plate 137. This organism produced spores rapidly. It was not possible to get motile growths by transplanting from one surface growth to another in agar, spores formed almost as rapidly as the new growth appeared. The only way we were able to get the true growth, one that would show flagella in staining, was from a fresh bouillon culture streaked on 1½ per cent agar, and then from a six or eight hours' growth. The anaerobes would not grow well in the bouillon at first, but afterwards we were able to get fairly good results. The bacilli have round ends 3 to 10 μ long

and 1 to 1.5 μ thick. (A μ is equal to one-twenty-five thousandth of an inch.) The rods grow in chains and form spores at the ends or near the center, in the latter case giving the cell a very plump appearance like a spindle. This is called a clostridium form. The cells are quite motile when young, and endowed with numerous flagella, as shown in Plate 134. The spores form rapidly and are thicker than the rod itself. They measure from 1.8 to 2.6 μ in thickness. When vegetating, the spore is ruptured at the end, and the young rod pushes out. One spore produces only a single rod. The rods lengthen and divide, and this is the manner of multiplication, true of all bacteria excepting the micrococci or round forms. This organism forms butyric acid, carbon dioxid and the hydrogen combines with sulphur and hydrogen sulphid is formed. This is the organism which softens the fiber and causes decomposition

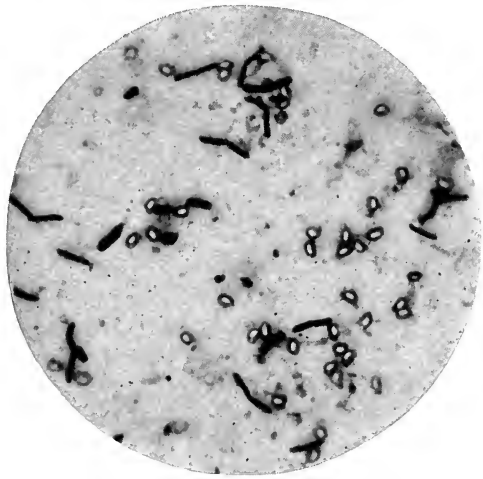


Plate 137

Pea Bacillus No. 2. Photomicrograph showing rods and spores. Culture from juice of peas, stained with carbol fuchsine. Slide preparation by the author. Magnified X 1,000.

of cellulose, in which case march gas or methane is sometimes formed. When starch is added to a culture medium and a growth of these bacilli is obtained, they may be stained with iodine and a beautiful blue stain is obtained. The cells of the bacteria will take in the starch and of course the blue reaction of iodine follows the staining. This is a beautiful experiment in staining and helps us to identify the germ as one answering the description of Bacillus Butyricus (Prazmowski) or Bacillus Amylobacter, a very common organism found in putrefying vegetable infusions in cheese, milk, etc.

The very fact that the anaerobic bacteria were present indicates that the process used (240° F. for 25 minutes) was much too low, because a temperature sufficient to destroy the spores of this species was not sufficient to destroy the spores of the other variety, as we found by actual test. We recommended that 250° F. be used for 25 or 30 minutes. The packer states in his letter that the process given these peas was the same as that successfully used for three years previously, and that the peas went through very promptly without any delays as formerly in the threshing; that conditions were 25 per cent better than they were in past seasons, and that the peas were younger and better than usual in quality for high grade goods. This would seem very mysterious to any one not acquainted with the biological characteristics of bacteria, but the reason we shall endeavor to explain.

When peas are allowed to stand, especially in piles, they become heated, and then follows what is called a "sweating process." This sweating is caused by bacteria starting with the lactic acid bacteria, which attack the carbohydrates, converting them into lactic acid, the fibre softens and the heat generated causes the spores of various species of bacteria to swell and vegetate. The aerobes and facultative anaerobes begin to vegetate first on the surface, thus utilizing all the available atmospheric oxygen, then the spores of the anaerobes soften and begin to vegetate, so that by the time the peas reach the sterilizing process all spores are softened, and many have no doubt vegetated. In the sterilizing process they are destroyed by a temperature considerably less than would have been required if the peas had been worked up quickly before the spores had started to vegetate. On first thought it would seem better to let peas stand a short time to allow the spores to soften in order to be sure of the sterilization. This, in fact, is the condition in a great many canneries, as the letter indicates there were numerous delays in the threshing during previous seasons, and those very same delays are the rule rather than the exception. It certainly follows that a process used successfully with peas handled in the manner just described will suddenly fail if used on strictly fresh peas. It must not be understood from this that serious delays are common in nearly all factories; we do not mean this, but there are only a very few who work up the peas without some delays. Where the delays are serious, there is formed sufficient lactic acid to cause sour peas.

No amount of sterilization will destroy the lactic acid when once formed, and the goods will be distinctly sour after the cans are opened, and in this case the liquor is frequently much clouded. The formation of lactic acid begins very soon if the vines or shelled peas are allowed to stand for any length of time, and this acid de-

stroys the fine flavor of the peas according to the amount present. The modern method of cutting the vines and threshing out the peas has some disadvantages over the old method of hand picking, because the juice is necessarily more exposed. But this is more than compensated by the many advantages in other ways, so that a better and more uniform quality may be secured if the arrangements are complete for taking care of the peas as rapidly as they are hauled in.

We may draw this conclusion from our study of the bacteria associated with peas. Absolutely fresh stock has more resistant spores than raw material which has been exposed for a limited time, and will therefore require a little higher temperature to insure perfect sterilization.

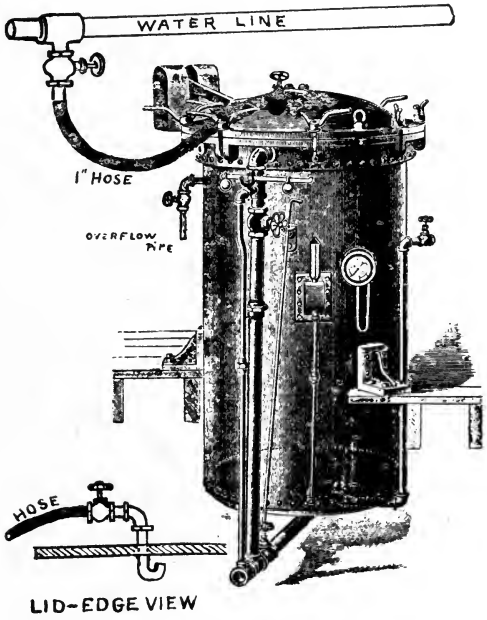


Fig. 34. Process Kettle

The quality of freshly canned peas will be very much superior even if the process is higher ,because no lactic acid is present; 250° F. for 25 to 30 minutes will be found quite sufficient, but if there is any evidence of scorching, this must be cut down, because it is not necessary to scorch any goods to insure sterilization. A perfect arrangement for chilling the cans is essential and the employment of alum after the blanching is to be recommended for its astringent action on the skins of the peas, which prevents their bursting during sterilization. Cloudy or muddy liquor will thus be

avoided, but all traces of the alum should be washed off with cold water before the peas are filled into the cans.

SPOILAGE OF CANNED PEAS.

It is a matter of wonder to us that some of the processes reported were sufficient in any degree. In reply to numerous letters of inquiry in the past, we have called attention to the fact that perfect sterilization of peas could not be accomplished under 250° F. for 25 or 30 minutes, and yet we have reports from various sources where the packers have used only 240° F. for as short a time as 16 to 18 minutes. Let us say that this temperature is entirely too low. While it might possibly be sufficient for some cans we predict that only a very few would remain in a perfect state of preservation for one year. Several canners have sent us cans taken from various piles and these we have placed in the incubator at 98° F., and in nearly all cases the cans have either swelled or turned sour after a few days. There are generally two different classes of organisms responsible for this spoilage. One class belongs to the anaerobic species; that is, the bacteria are able to grow only where atmospheric oxygen is entirely excluded or replaced by some other gas. These germs produce large quantities of gas which is malodorous, being a combination of sulphuretted hydrogen, phosphoretted hydrogen and methane. The pressure of this gas in the cans is so great at times that the seams burst, the ends blow out and the steel sheet is rent. Some of the cans sent to us arrived entirely empty, the contents having escaped in transit.

The other class of germs is aerobic, or facultative anaerobic. By this we mean the bacteria are able to grow in an environment devoid of oxygen but their nature is to grow in the presence of oxygen. These germs produce chemical changes in the peas which are far more dangerous than those produced by the other kind, from the fact that they do not form any gases excepting small quantities of sulphuretted hydrogen, which is absorbed by the fluid of the contents. Where peas are spoiled by these bacteria, they give no evidence of the fact until the cans are opened. The liquor is generally muddy and the peas are intensely acid. The natural sugar or carbohydrates is converted into simple and complex fatty acids. There is a large number of different species included in these two classes of bacteria just described. The general characteristics of all are similar. There are some biological or morphological distinctions by which we are able to identify them and note the peculiar changes brought about in the certain cans.

The following letter was received from a prominent canner of peas:

National Canners' Laboratory, Aspinwall, Pa.:

GENTLEMEN:—We are sending you today by prepaid express six cans of new pack of peas, four cans from early pack and two cans from late pack. We have had complaint concerning cloudy liquor on these goods and we would like you to inform us the cause of this, etc.

Hoping to hear from you as early as possible and thanking you, we remain, Yours very truly,

_____.

When these peas arrived we opened them carefully and examined the juice under the microscope. The early varieties seemed to be the only ones affected. The liquor on the large varieties was perfectly clear. In the other case the peas were very much broken and the protein matter was cooked up with the juice so that in some cans it presented the appearance of pea soup. It was evident to my mind that these peas had been over-processed, but I cannot say what process was used because none is mentioned in the letters received. There are several conditions from which may result muddy liquor and bursted peas, and it is sometimes difficult to know just what the cause is, without being perfectly familiar with every step in the process of manufacture. For instance: If the shelled peas had been allowed to stand for any great length of time in baskets before blanching, it is quite likely that the skins would suffer from the action of bacteria. We know that certain bacteria have the power to soften fiber, and the spores of this very species are always present on the skins of peas, having found their way there in the machines which do the hulling. Now, in case shelled peas were allowed to stand, even over night, when the temperature was quite warm, this decomposition of the fiber would likely take place. It is quite easy to understand, therefore, that the skins would not be able to withstand the pressure ordinarily given peas in sterilization; they would burst and cloud the liquor. The same difficulty might arise from peas that had been filled into the cans, if the cans were allowed to stand for a long time, on account of breakdowns in the machinery, or overcrowding, but generally this bursting of the peas is due to over-processing. This case was so extremely bad that there is little doubt of this solution being correct.

As we stated in a previous chapter, the processing should be just sufficient to keep the goods without danger of bursting the peas or imparting to them a scorched taste or odor. If a proper chilling apparatus be employed and alum used in the blanching process to toughen the skins of the peas, which it does by its astringent properties, there will be very little danger of peas bursting or having a scorched taste, unless the sterilization is pushed entirely too

far. We have found for all practical purposes, that 250° for 20, 25 and 30 minutes, according to the sizes, will generally give pretty good results. We are aware that this process may be reduced somewhat in certain sections, but not very much, without great danger of spoilage.

E. W. Duckwall, Aspinwall, Pa.

DEAR SIR:—We sent you some days ago samples of peas that we cooked 16 minutes at 240°. These you reported as being all O K.

We are sending you today six tins of peas, three that are bulged and three cooked same as the other cans sent you. Kindly advise if the three tins bulged are swells or leaks, also if the three cans that appear all right are all OK. In our opinion the three cans look like swells, as we have been unable to detect any leak in the cans. However, there may be in the figure stamped on the cap or possibly in the impression.

Very truly yours,

_____.

E. W. Duckwall, Aspinwall, Pa.

DEAR SIR:—Your favor of the 9th at hand, same being in regard to the six cans of peas sent you Aug. 2d. We became convinced shortly after we sent these peas that we had swells. We have canned peas at this place for seven years. We have several years cooked our Alaska peas as low as 15 and 16 minutes at 240. Our sweet wrinkle peas we have cooked anywhere from 18 to 22 minutes at 240, and we never have had any swells before. Our Alaska pack this year were all cooked 18 minutes at 240. The Admiral peas this year we cooked nearly all at 240 for 18 minutes. We cooked a small amount 16 minutes at 240. The first cans we sent you were from the first of our 16 minute cook, and these you reported free from bacteria. Our Alaska's this year have no swells in them. We have now nearly completed piling our Admiral peas and have taken out about 10,000 swells. The small sizes seem to have more swells than any of the others, the third sieve or what we call sifted peas having the most. We are at a loss to know how soon it would be safe to begin shipping from these goods, and whether swells will continue to show up in the goods from now on. We have never been able to cook peas more than about 20 minutes at 240 and get a clear liquor. Answering your question as to a preservative, would say that we have never used any in our peas.

Yours truly,

_____.

We were very much surprised on reading these letters that the parties had processed their goods successfully, as they claim, for a number of years at 240° F. for only 16 to 18 and 22 minutes, and it is not surprising that the loss is so great, as the letter states that up to this time they have taken out about 10,000 swells.

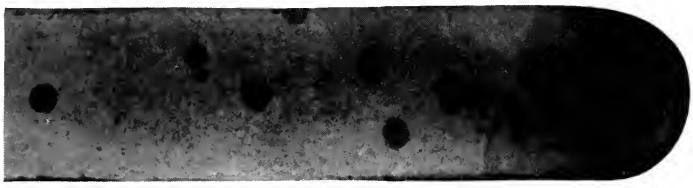


Plate 138

Photograph of the colonies of the anaerobic species described in Plate 139. This is an agar slant and the colonies are growing on the surface. The rings are easily seen. See text. Magnified 2 times.

I immediately examined the two cans left in the incubator and found one of them swelled. In my previous letter to these parties I asked them if they had used any preservatives. I did not make an analysis to determine if preservatives had been employed, and

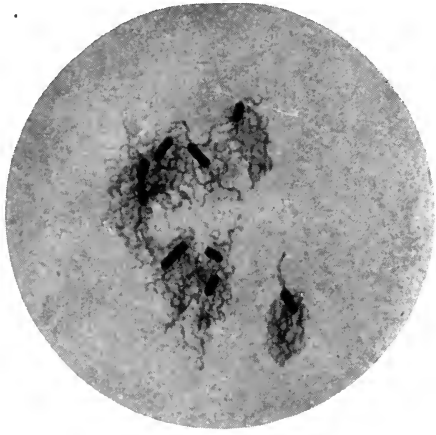


Plate 139

Photomicrograph of young vegetating rods of the anaerobic pea bacillus found in can of swelled peas. It forms terminal spores in three days. The young cultures are actively motile, being endowed with numerous very curly flagella so characteristic of anaerobic varieties. This specimen was stained with difficulty. Magnified 1,000 diameters.

this was unnecessary, because their last letter states that none had been used. I wrote these parties at once that I feared their spoilage would be very great, because I did not see how it would be possible to keep these goods from spoiling in hot weather.

The bacteria which are usually responsible for the spoilage of peas grow best in a temperature between 80° and 98° F. It is possible to reprocess the cans at 240° F. for fifteen minutes.. All cans which have started to spoil will swell slightly in the process and the ends will draw in slowly, and they may be separated from the good. All cans which are infected with only a few bacteria can be saved by reprocessing. I set about to determine what organisms were responsible for this spoilage. I inoculated several tubes of nutrient bouillon; also several test tubes of agar slants. I also streaked the surface of several Petri dishes containing nutrient agar. Some of the tubes of bouillon I sealed in the anaerobic apparatus and produced that condition by means of Pyrogallic acid

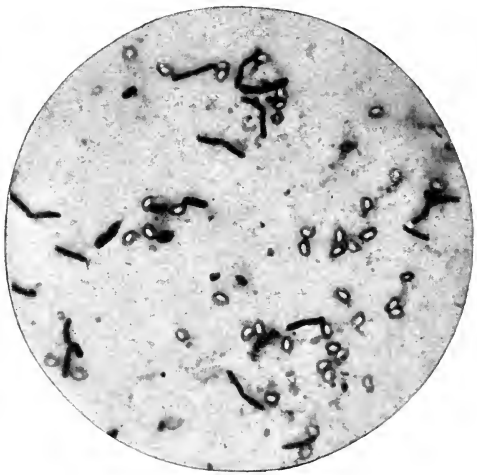


Plate 140

Photomicrograph of the spore bearing rods of the anaerobic pea bacillus found in a can of swelled peas. The spores are terminal and greatly resemble B. tetanus. This photograph shows how the old cells dissolve in the surrounding fluid. Magnified 1,000 diameters.

and a weak solution of sodium hydroxid. When the acid and the alkali come together, all the oxygen in the apparatus is absorbed, which leaves the tubes containing bouillon and agar in an anaerobic condition. The bouillon became cloudy within a few days. Cloudiness in the bouillon always indicates the presence of bacteria. Colonies also made their appearance on the agar slants. These we examined carefully and found that there were two kinds. One kind was an obligative anaerobe. The other kind was a facultative anaerobe. The first kind is able to grow only where oxygen is entirely excluded; the second kind is able to grow in the presence or absence of oxygen.

DESCRIPTION OF THE COLONIES OF THE ANAEROBES.

The colonies were round and scalloped; of a brownish color; the surface was granular and there was a light-colored zone forming a ring near the outer edge. This was seen when magnified 60 diameters. The natural color of the colony was white, and by transmitted light was gray with a blue center, white periphery. The surface looked moist and showed a distinct bluish colored circle in the center. Colonies are slightly elevated, measuring from 3 to 6 millimeters in diameter. After transplanting to other slants, we were able to get a superficial growth of these bacteria, and to stain them for flagella, as the accompanying plate will illustrate. The flagella are organs of locomotion, and like all motile anae-

Plate 141

This is a photograph of the streak growth on agar in Petri dishes. The streaks were made up and down. The outgrowths on both sides are characteristic. No other organism has a growth just like this. This growth is from a streak made 24 hours previously and incubated at 98 degrees F.

robes, the bacteria are very much curled. In various views of the slides which we stained there were large twisted bodies, which are called giant whips. Many cultures of bacteria have the faculty of forming giant whips. Just what these bodies are has not been fully established, but it is thought that they are formed by numerous flagella which have fallen away from the germs and become twisted together. This organism produces a wonderful amount of gas, having a distinct odor of sulphuretted hydrogen. The water of condensation, which always forms at the bottom of an agar slant, fermented very freely and the escape of gas was evident by the large number of gas bubbles. We grew this organism in milk

Bacillus Mycoides

Origin.—Widely distributed in earth; found also in river and in spring water.

Form.—Rather large rods, with slightly rounded ends; these are thicker than the hay bacillus. Threads are common.

Motility.—It has a slow motion.

Sporulation.—Forms small median spores.

Anilin Dyes.—Stain readily.

Growth.—Rapid.

Gelatin Plates.—The colonies somewhat resemble fine branching rootlets. At first they are round and dark, with bristly borders, but they subsequently branch out through the gelatin, which is slowly liquefied.

Stab Culture. This is characteristic, the growth developing along the line of inoculation and from this threads penetrate or radiate into the surrounding gelatin. The growth being more rapid at the top than in the lower parts of the tube, the result is that it has the appearance of an inverted pine tree. Subsequently the gelatin is completely liquefied, the bacterial growth accumulating on the bottom and the liquid above becoming clear, with a thin scum on the surface.

Streak Culture.—On agar, a grayish growth is formed which spreads outward from the streak, often giving it an appearance resembling the centipede. On potato, it forms a slimy, whitish growth which contains large numbers of spores.

Oxygen Requirements.—Aerobic.

Temperature.—It will grow at ordinary temperature, and in the incubator.

Behavior to Gelatin.—Liquefies slowly.

Pathogenesis. It has no effect, not even in large doses. Experiments are now being made on patients suffering with tubercle bacilli in the lymph system. Pure cultures of Bacillus Mycoides injected into the lymph glands seem to antagonize the multiplication of tubercle bacilli.

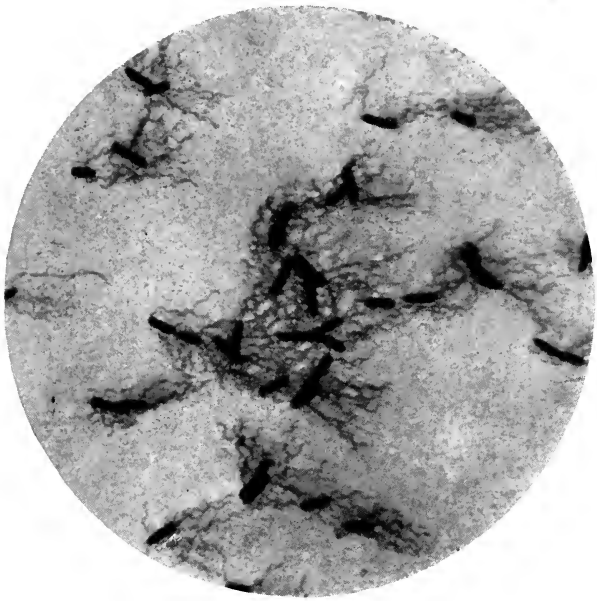


Plate 142

Photomicrograph of a slime producing bacillus isolated from peas. It was slowly motile, able to grow in the presence or absence of oxygen, produces acids without formation of gas. It is closely allied to Bacillus Mycoides. Owing to the formation of slime, the flagella were difficult to demonstrate, the slime was precipitated with chloroform, and the flagella were stained with a mordant and carbol fuchsine. Magnified 1,000 diameters.

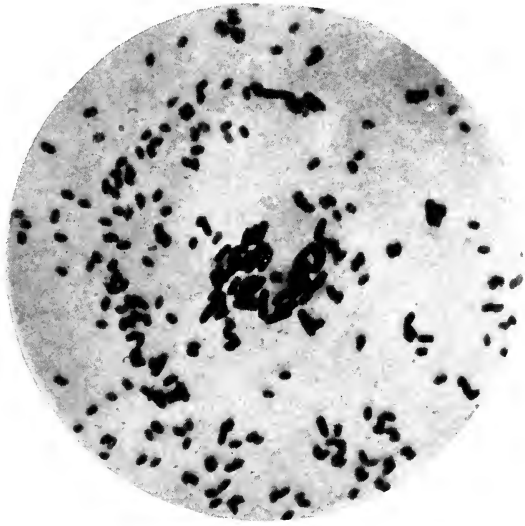


Plate 143

Photomicrograph showing rods and spores of the bacillus described under Plate 142, a facultative anaerobic germ isolated from canned peas. The spores are extremely small and located at the center of the rods. Stained by carbol gentian violet. Magnified 1,500 diameters

made blue by tincture of litmus, and within 24 hours the litmus had turned red and the milk became curdled. After 24 hours more the curd was dissolved and a heavy precipitate formed at the bottom of the tube. These organisms formed large terminal spores and grow more freely than the Butyric Acid bacilli, so named by Prazmowski. The spores of these bacteria were quite resistant to heat. They are able to withstand a temperature of 240° F. for about 10 minutes in test tubes, and in No. 2 cans of peas are not destroyed under 25 to 30 minutes at 240° F. We were able to produce some swelling of the cans in peas that we had in the laboratory by inoculating cans with the spores. The germs do not grow well at ordinary temperature of 50° to 70° F., and do not grow at all between 35 ° and 45° F.

DESCRIPTION OF THE AEROBIC VARIETY.

The aerobic variety seems to correspond pretty closely with bacillus mycoides. The colonies were round, and of a bluish transparent cast. Under the microscope the edges are slightly scalloped; a smooth center, slightly yellow, becoming thin and transparent at the periphery. The natural size of the colony is about 1 to 4 millimeters; extremely slimy, so that a needle dipped into the colony will carry a slimy thread for several inches on removal. We had a great difficulty in demonstrating the flagella. In this respect the germ resembles Bacillus Mycoides. The streak on agar developed quite rapidly and grew down into the medium. Along the center scaly folds would form and from the edges would be distinct branches typical in every way of the Root Bacillus. Its identity therefore is established somewhere between Bacillus Vulgatus and Bacillus Mycoides. We stained the flagella with difficulty. We took the growth as young as possible, but were unable to get any preparation entirely free from slime. Some writers have declared that Bacillus Mycoides has no flagella or organs of locomotion, and after several futile attempts to demonstrate their presence by the regular method, it seemed probable that the flagella were absent. In hanging drop cultures, however, we could see that the bacilli were motile, having distinct serpentine and oscillating motion. On every cover glass the slime would form a very thin layer, sufficiently heavy, however, to obliterate the flagella, so we adopted another method of staining. We inoculated one cubic centimeter of water with a young culture; then added one cubic centimeter of chloroform, agitating the two together for some time. The chloroform dissolved the slime and carried it down to the bottom. From the water above the chloroform we were able to get some very fair preparations for flagella staining. The accompanying plate shows the result of this work. This organism has a very small spore

centrally located in the rods. When growing in peas there is no formation of gas. The carbohydrates are decomposed into fatty acids. A small quantity of H_2S or sulphuretted hydrogen is formed, but not enough to cause swelling of the cans. The spores of these bacilli are more resistant to heat than the former organism, and are therefore more inimical from the fact that complete chemical decomposition may take place without being apparent. The can will look perfectly natural and will not spring, this showing that the vacuum is still present.

CHAPTER XIII.

Tomatoes

Character of Tomatoes Raised in Different Localities. Method of Canning. Cold Packed Tomatoes. Suggestions. Laboratory Work. Various Bacteria Found in Leaky Cans of Tomatoes. Sour Tomatoes Due to Souring Before the Sterilizing Process; the Cause and Remedy. An Attempt to Pack Tomatoes in a Vacuum Jar Without Sterilization; Cause of the Spoilage. Bacteria the Cause of Tomato Black-rot Disease. Uneven Temperature in the Process, Resulting in Loss.

Tomatoes raised in one locality differ very much in character from those raised in another locality. The tomatoes raised in our northern states are more meaty and contain more sugar than those raised in the Valley of the Mississippi and the extreme eastern coast of the United States. The sweeter tomatoes are the best for canning purposes, while for making tomato catsup, chili-sauce and other condiments of this character, those which contain more acid are more desirable.

It is difficult to say at what date the canning of tomatoes in this country can be set down accurately. There is a record of William Underwood having canned tomatoes in glass as far back as the year 1820, but the business did not grow to any considerable extent until about 1875-80. There were a few scattered factories canning tomatoes between the years 1860 and 1875, most of them in the neighborhood of Baltimore. The peeling of tomatoes was done by hand and there have been no machines perfected for this work up to this day. The filling of tomatoes into cans, capping, tipping, etc., were done by hand. Today most of the filling is done by machinery and the capping, of course, is put through rapidly by the automatic capping machines.

Probably the best means of canning tomatoes is by what is known as a "cold process," that is to say, the tomatoes are filled into the cans after they are peeled and are not heated prior to the final sterilizing process. The old style of canning tomatoes was to fill the cans, then give them a heating in boiling water with the vents of the caps open; afterward they were removed and the vent holes soldered up, and then the cans were subjected to a sterilizing process of about thirty minutes at 212° F.

Tomatoes are not difficult to keep; all that is required is rapidity. This is absolutely necessary if one desires to can cold-packed tomatoes. They must be worked up very rapidly so that no bacteria will start fermentation. Fermentation of tomatoes may be due to several kinds of micro-organisms, but principally to wild yeast molds, acetic acid, and lactic acid bacteria. In this process of decomposition there is a gas liberated, carbon dioxid. If this gas is liberated in any quantity, it will prevent the cans from collapsing after the sterilizing process. In this case, it would be impossible to turn out fine goods unless the cans were vented. I would like to impress this point, namely, that if cold-packed tomatoes are to be produced, there must be absolutely no fermentation of the tomatoes in any process prior to sterilization. Fermentation sets in very rapidly after the tomatoes are peeled, particularly in weather where the thermometer registers between 80° and 90° F. They will ferment sometimes in the buckets of the peelers, especially where the peeler is rather slow in turning out her work. It is better to have small-sized buckets for the peelers and then keep the tomatoes worked up very closely.

Much depends on the scalding apparatus. If the tomatoes are not properly scalded, fermentation is much more likely to follow than if the outside of the tomato simply is scalded. If the water is not kept at least 212° F. the tomatoes will be cooked through to the center. To simply scald the outside of the tomato, leaving the inside cool, is the ideal method of loosening the peels. In the sterilizing process, cold-packed tomatoes can be kept all right with a process of 35-40 minutes at 212° F. As a rule, the packer can judge whether his tomatoes are perfectly sterilized by examining the seeds. There is a gelatinous envelope around the seed in raw tomatoes and the cooking must be sufficiently prolonged to loosen this envelope. We have seen the seeds from canned tomatoes planted and some of them sprouted. All such canned tomatoes will swell; sometimes when no bacteria are present, there will be sufficient evolution of carbonic acid gas from the seeds themselves to cause the spoiling of the goods. Tomatoes are acid by nature and cans made for them must be well soldered. The smallest possible leak in the can will be much enlarged. In some cases where there is no leak at first the solder may be strained in the sterilizing process or by rough handling, and the acid juice of the tomato will work through. A great many of the spoilage cases of tomatoes submitted to the laboratory were due to leaks. In the following pages we will give some cases in detail.

The year 1903 was the largest in the history of the canning business for tomatoes, the packing amounting to ten and one-half million cases.

A canner wrote as follows: "We have a condition this year with our tomato pack that we have never had before, and we are trying to locate the cause. Nine-tenths of them seem to be all right, but about one in every ten is sour, a fermented sour, but the can is not swelled in the least. They cannot be located without cutting the can. Can you tell us the cause and give us a remedy; and is there any danger of the per cent getting larger when warm weather sets in? We are sending you today a case of the tomatoes that we told you were sour without any cause that we could determine. There are only about 10 or 20 per cent of them sour.

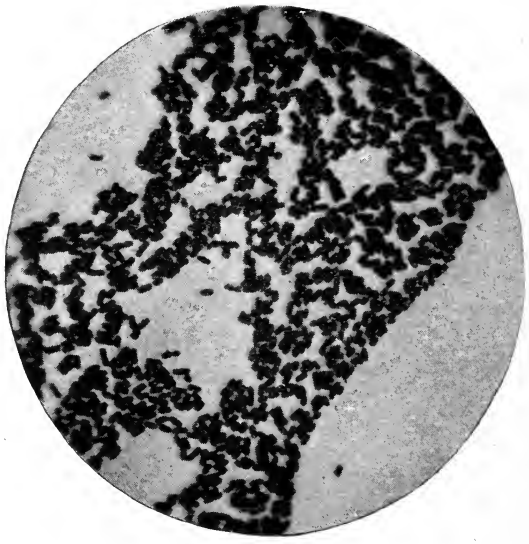


Plate 144

Photomicrograph of Bacillus Acidi Aceti or Mycoderma Aceti or "Mother of Vinegar," showing short dumb-bell rods, large lemon shaped and drumstick involution forms. Produced acetic acid in tomatoes; isolated by plate culture method; stained with fuchsine and mounted in xylol balsam. Magnified 1,000 diameters.

The can that is wrapped in tissue is one that we opened and found to be sour, and then sealed it again. We have had canned tomatoes here for years and have never seen anything like it before. We do not know the cause. As we put up a pack of 8,000 cases and as we kept no record of each day's pack, it would be hard to tell just what were the exact conditions under which the case which you got were packed, but I think I can tell you near enough what the conditions were all through, so you can determine what is the matter. The sample you got was taken from the early packing, where most of the trouble seems to be. The tomatoes as they came in were quite ripe, and sometimes they would stand on the platform in crates for

two or three days, but they seemed to be in good condition, i. e., there were not any rotten. They were scalded, peeled and put into cans reasonably quick. At times I presume there were tomatoes that stood for 30 minutes after they were peeled before they were put into the can. I hardly think that they ever stood longer than 30 minutes, as we were afraid to let them stand around after they were peeled. There were very few that stood longer than fifteen minutes before they were put into the can. At times leaks would be allowed to stand for an hour or two before repairing. I notice, however, that there are sours among those that were never patched. We processed 30 minutes in water at the boiling point, but it may be possible that the water got below boiling point sometimes, although we were careful about keeping the water hot.

When the samples of sour tomatoes arrived we found that quite a number of cans were simply cap and tip leaks, but there were other cans which were very sour, yet showed no signs of leaks. We made bacteriological tests of all the cans which appeared to be sound, and in no case did we find any living bacteria in these cans. From the leaky cans, however, we isolated two kinds of bacteria which, when transplanted into good cans produced the same acids and aromatic flavors peculiar to the sour cans of tomatoes, showing that the conditions around the factory were such as to endanger the goods at all times.

The liquid in some of the leaky cans had turned to vinegar, and this had been attached by another organism, which we shall presently describe. The acetic acid bacteria are little rods having a slight constriction in the middle, giving them a dumb-bell shape when highly magnified. These bacteria are subject to complete change of form and this peculiarity is technically called involution form. Plate 144 will show examples of all forms from the small typical rods, to swelled lemon shaped, club shaped and other forms so frequently seen in the mycoderma aceti or "mother of vinegar." When these germs are alone in their work, only acetic acid is formed, but when other organisms are present the acetic acid is changed and flavored with unpleasant aromatic substances, such as volatile fatty acids and ethers.

We isolated a chromogenic bacillus, which produced a reddish violet pigment in the agar. When transplanted into tomato juice it produced a deep red color and formed a pellicle on the surface. It is a slowly motile organism with very fine flagella, running out in gentle curls from the whole surface of rod. Our plate is magnified about 2,000 diameters and this is about four million times magnified.

From the pellicle we obtained the spores of the bacillus and were not able to destroy them by boiling. They do not grow readily on pure tomatoes and seem to demand acetic acid to get a

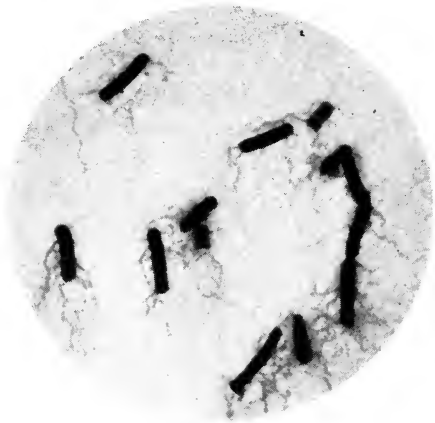


Plate 145

Photomicrograph of Bacillus X, a chromogenic motile bacillus, isolated from a can of spoiled tomatoes where the acetic acid bacteria were also present. It has numerous hair like flagella and moves in a slow wabbling motion. It produced a dark red pigment. Stained by our special method, from Agar culture very young. Magnified 2,000 diameters.

start, so this accounts for their not being generally found on tomatoes unless other organisms have worked out chemical changes, or produced substances favorable for their propagation. If we had to contend with this bacillus generally, our tomatoes would require

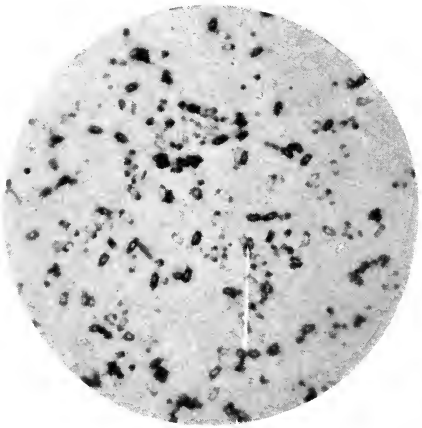


Plate 146

Photomicrograph.—Spores and rods of Bacillus X, isolated from sour tomatoes. The whole rod seems to become a spore, the cell contents are apparently surrounded by the cell membrane almost the entire length. This afterwards contracts and an oval spore is set free. Stained with carbol fuchsine. Magnified 1,000 diameters.

250 degrees F. for perhaps 25 minutes to destroy the spores. This would, of course, cook the tomatoes too much and it would be a problem to successfully pack them. If, however, we observe the rule, to work up the raw material as fast as it is received, we need have no fear of this difficulty.

CONCLUSIONS.

After investigating carefully the samples of sour tomatoes sent me I submit my report. There are quite a number of leaks among the cans in the capping and tipping. There are no living bacteria in any of the good cans or the cans which are sour, excepting the leaks, therefore there will be no further souring of the goods. The sour tomatoes were sour before they were processed, probably before they were scalded, the acid having formed in them and still remains in the finished goods. I would advise you to work your tomatoes up quickly, or if unable to do this at all times, provide a way to make them into tomato pulp for catsup, etc. If you have much trouble with breakdowns, try and make such improvements in your mechanical apparatus as will preclude the possibility of souring. Have your scalding water boiling hot—that is, hot enough to scald the skins off the tomatoes and still leave the inside of the fruit cool. In this way you will avoid souring after the peeling, to a great extent. I met with a case similar to this one in 1891 which lead me to take up the study of bacteriology in connection with canning.

If you have any doubts of your sterilizing apparatus, look into the matter carefully and arrange your system in such a way that no mistakes will be made at that point next season. This case has nothing to do with sterilization; the souring occurred before the tomatoes reached the process. I want to call your attention again to poor soldering, and would advise you to take steps to improve in that work. A great deal of your spoilage is no doubt due to imperfect work.

TOMATOES PACKED IN A VACUUM JAR.

The sample of spoiled Tomatoes was fermenting and the escaping gas had made an opening through the wax. A microscopical examination of the juice showed large numbers of yeast-like forms which we mounted on a slide. Plate 147 illustrates their appearance. The dark cells are the oldest, and from these others have grown out from all sides, first making their appearance as very small excrescences, then gradually filling out, soon attain the same size and appearance as the mother cell, and in like manner these give rise to buds as before, until quite a little bunch will be seen en masse.

In order to determine just what these were we streaked a number of Petri dishes containing tomato agar. Tomato agar is simply filtered tomato juice to which one and one-half per cent of Agar-agar has been added to make a solid culture medium or jelly

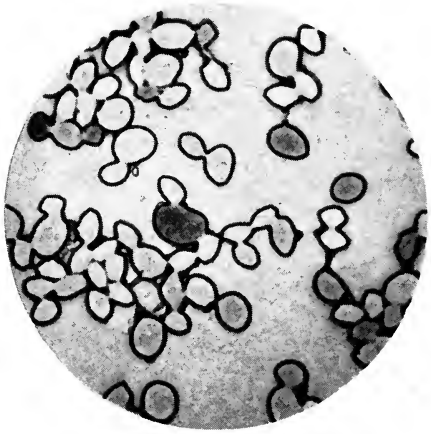


Plate 147

Photomicrograph of the building Conidia of Mucor Mucedo, obtained from a jar of spoiled tomatoes undergoing fermentation. These conidia have the power of setting up a fermentation similar in many respects to that of the yeasts. In this manner of growth Mucor Mucedo looks very much like the brewers' yeast Saccharomyces cerevisiae. Magnified 1,000 diameters.

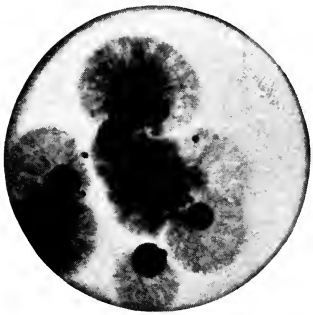


Plate 148

Photograph of a Petri dish which had been streaked with the juice of fermenting tomato. The moss-like growths are the Mucor Mucedo growing in presence of atmospheric oxygen. The large round spots are motile bacteria growing in colonies. The small round spots are Acetic Acid Bacteria in colonies. This is the method we employ to isolate the various bacteria found in spoiled goods. The Petri dish contains a medium of tomato juice and Agar jelly.

culture. In two days our dishes had very good growth, and Plate 148 will illustrate their appearance. The moss-like growths are mycelia of the mold plant Mucor Mucedo, which is a fungus belonging to the order of Hyphomycetes, a higher type in the botanical

classification than the bacteria. The round dark spots are colonies of bacteria, which we will describe later on. Plate 149 is a magnification of Plate 148 of about four diameters, showing more distinctly the moss-like growth. The very small dark spots through-

Plate 149

Photograph of Plate No. 148 magnified four times. In the moss like growths of the mold Mucor Mucedo there are a number of very small dark spots. These are the first pods which are borne on very delicate hair-like stocks. The round pods contain the little seed forms called conidia, which are shown in Plate 147.

out the moss-like growths are the fruit pods, which grow on very slender—hair-like—stems or sporangia. There are a large number of these, which soon attain a height of an inch or more under favorable conditions, and at the top of each is a small round pod or cell which contains a vast number of tiny, round, shot-like seeds, called conidia, each one of which is able to start a new mold plant.

Plates 150 and 151 are good illustrations of the microscopical appearance of these cells containing seeds conidia, under a magnification of 800 diameters. These very delicate forms of vegetable life are so tender and sensitive that we cannot stain and mount them in the usual manner, consequently the photography is quite difficult, since they must be mounted alive in glycerine, fixed without heat, and unstained. The Mucor is almost transparent, and consequently a negative giving good contrast cannot be obtained. In the two plates we have part of the mycelium very much interlaced, as it is naturally, and scattered throughout are the round fruit pods containing the seed or conidia.

Plate 150

Photomicrograph of Mucor Mucedo in the living state, mounted in glycerine. The round pod in the center contains the seed forms or conidia. This pod is ripe, ready to burst when the conidia are carried by water or air, ready to start a new mold plant or to set up fermentation according to the conditions in which they are thrown. Magnified 800 diameters.

The conidia have peculiar morphological characteristics, being able to assume two distinct biological characters marked by the manner of their reproduction and the chemical changes wrought, especially when compelled to live in a partial or complete anaerobic environment. In this condition their appearance and vegetation resemble the Blastomycetes or yeasts so closely that differentiation is difficult, unless we resort to the plate culture method, as was done in this case. Plate 147 illustrates the growth of Mucor Mucedo when submerged in nutrient tomato juice, where its supply of atmospheric oxygen is cut off, while Plates 148 and 149 show the natural growth when the conidia are able to obtain oxygen from the atmosphere.

When growing in an anaerobic condition the supply of oxygen (which mold demands in large quantities) is obtained from the various molecules of vegetable matter, and the carbohydrates,

setting free carbonic acid gas in considerable volume, by which reaction, alcohol, succinic acid, glycerin, volatile fatty acids and others are formed, which in their turn are attacked by various kinds of bacteria, most commonly by the acetic acid group, but frequently by motile putrefactive organisms, which produce disagreeable aromatic compounds.

In Plates 148 and 149, we notice the dark round spots among the mold filament. There are colonies of bacteria, and there are two kinds, one of which is the acetic acid bacillus, described previously. Plate 153 is a bacillus much resembling Megatherium in its spore formation and the arrangement of its flagella or

Plate 151

Photomicrograph of Mucor Mucedo, showing the seed pods containing the conidia. These pods are not yet ripe, consequently the conidia are not yet perfectly formed. This specimen is living and mounted in glycerin for microscopical examination. Magnified 800 diameters.

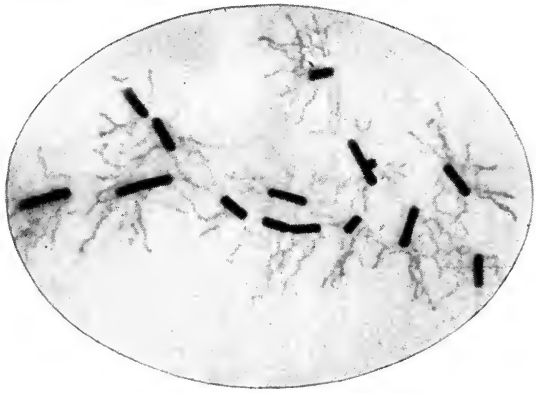


Plate 152

Photomicrograph of the Tomato Bacillus, a slowly motile, aromatic bacillus, which destroys tomatoes by the unpleasant flavor it produces. This specimen was obtained from a young growth on tomato agar, and the demonstration of the flagella was done by author's special staining method. Magnified 1,000 diameters.

organs of locomotion, but far more difficult to stain. The spore formation is peculiar, as shown in Plate 154. The spore occupies nearly the whole length of the cell, but gradually becomes smaller as the cell membrane is dissolved in the surrounding fluid. After this organism has done its work, the tomato juice has such a disagreeable odor that nothing can be done with it. Ordinarily, tomatoes which have simply undergone fermentation may be made into very good catsup, but not the best quality.

BLACK ROT OF TOMATOES.

We received by express a tomato, one side of which was completely diseased by what is known as black rot disease, and the following letter will explain:

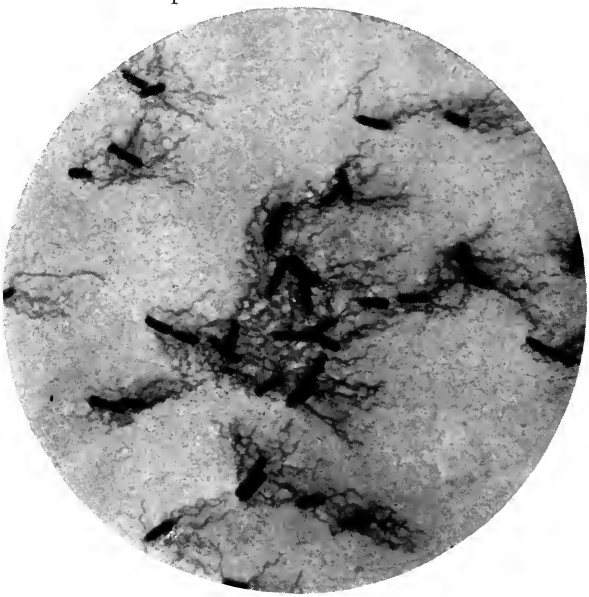


Plate 153

Photograph of the Tomato Bacillus, as shown in Plate 142. The spores are quite long when forming within the rods, occupying nearly the whole cell. After being set free they become smaller and the membrane becomes thicker. Stained with carbol fuchsine mounted in Xylol Balsam. Magnified 1,000 diameters.

"I send you under separate cover sample green tomato. A great many of the tomatoes on our vines present the same appearance as this sample. Is this not caused by the tomato louse? Your opinion will be appreciated."

The cause of the black rot is not due to the tomato louse, as the letter suggests, but to a bacterium. We made cultures of this organism from the tomato, and were successful in getting fine growths on nutrient agar in Petri dishes. In order to be certain that our culture was the right organism, we inoculated several to-

matoes with a loop full of the bacteria from the pure culture, and were successful in transplanting the disease. We found, however, that we could not communicate the disease to perfect tomatoes without puncturing the skin. When the bacteria were simply spread over the surface of the skin, they remained dormant or dried up without inducing the disease. We are at a loss, however, to know how to apply a remedy for the black rot in the patch of tomatos. We have noticed that this disease is more frequent in either extremely dry or in extremely wet weather. In dry weather the tomatoes are frequently attacked by insects and the bacteria which are responsible for black rot thus gain an entrance through the perforated skin. Sometimes after a rain the sun will come out very brightly and the skins will crack open under the influence of the heat, thus affording a means of invasion by bacteria.

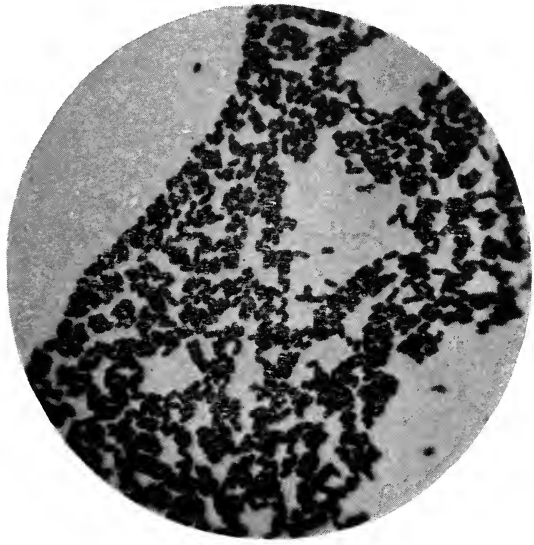


Plate 154

Photomicrograph of Bacillus Acidi Aceti or Mycoderma Aceti or "Mother of Vinegar," showing short dumb-bell rods, large lemon-shaped and drumstick, involution forms. Produced acetic acid in tomatoes; isolated by plate culture method; stained with fuchsine and mounted in xylol balsam. Magnified 1,000 diameters.

In extremely wet weather the plants are sometimes knocked down and the tomatoes rest on the wet ground where they are attacked by worms and various insects and bacteria may set up the disease through the perforations thus made in the skin. The black rot disease sometimes works its way entirely through the tomato, destroying it. Frequently only a portion is affected, and this is removed, of course, when the tomatoes are peeled. When tomatoes are intended for catsup, all such diseased places have to be cut with a knife, because black rot disease will permeate the whole batch of pulp, and black specks in the goods will result. While we are not

able to suggest any means of destroying the bacteria which are responsible for such widespread destruction of tomatoes as we sometimes see, it is interesting to know just what organisms are responsible, and gives us a means of studying the problem of overcoming these losses. The accompanying plate gives a microscopic view of the black rot bacilli as they appear when magnified 1,200 diameters. The colonies of this germ are of a slightly yellow color, even bordered. The streak culture on agar is somewhat lustrous and yellowish in color, after a time becoming darker, deepening into almost a brown. The organism is not motile, and so far as we were able to study its characteristics, it does not form spores. We intend to study the morphological and biological characteristics of the tomato black rot bacillus, and endeavor to find some means of protecting the maturing tomatoes in the patch when this disease is rampant.

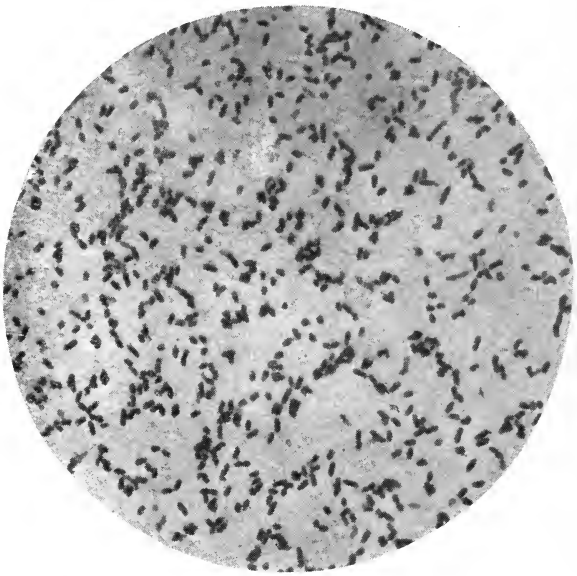


Plate 155

Bacillus of Tomato Black Rot disease. This is not a motile organism, and no spores have been observed. This is a photomicrograph obtained from a fuchsine stained preparation of pure culture of the bacilli on Agar. Magnified 1,200 diameters.

A CASE OF LEAKY CANS.

The case of tomatoes arrived at the laboratory and a pretty thorough examination was made of every can and particularly the solder. Some of the solder, when viewed through a sixteen millimeter objective, had a honeycombed appearance. Solder made in the proportion of thirty tin and seventy lead is not fit for either can making, capping or tipping. Solder used in can making ought to

be about half and half and that used for capping and tipping forty tin and sixty lead. Solder made with seventy per cent lead would necessarily be much weaker than that which contained a greater per cent of tin, and then again such solder is liable to impart traces of lead to the canned product. A complete report of these cans is here appended:

FIRST CAUSE OF ALL SPOILAGE IS LEAK IN CANS.

1 No. 3 can marked "P" perforated tin plate.
1 No. 3 can marked "O" seam leak.
1 No. 3 can marked "O" seam leak.
1 No. 3 can marked "O" seam leak.
1 No. 3 can marked "P" seam leak.
1 No. 3 can marked "P" seam leak.
1 No. 3 can marked "P" seam leak.
1 No. 2 can marked "P" seam leak.
1 No. 2 can marked "P" cap leak.
1 No. 2 can marked "P" seam leak.
1 No. 2 can marked "P" seam leak.
1 No. 2 can marked "P" seam leak.
1 No. 2 can marked "P" top leak.
1 No. 2 can marked "P" seam leak.
1 No. 2 can marked "P" broken plate top seam.
1 No. 2 can marked "P" seam leak.
1 No. 2 can marked "P" seam leak.
1 No. 2 can marked "P" seam leak.
1 No. 2 can marked "P" seam leak.
1 gallon can, seam leak.

Cultures were made of the bacteria found in some of the doubtful cans and in every case the bacteria were not spore-bearing, but belonged generally to the acetic acid varieties. Pure cultures were made of these and a ten per cent alcohol solution was inoculated with some of the culture, which rapidly attacked the alcohol and converted it into acetic acid. The germs which were found in these cans are freely distributed in the air and no doubt gained entrance through the leaks. The time which is given in the letter for two and three-pound tomatoes is really more than is necessary. Thirty-five to forty minutes for No. 3 tomatoes ought to sterilize them perfectly. *Tomatoes need not be exhausted.* They will keep all right if the process of sterilization is sufficient. It is customary to add about five minutes' more time for cold-packed tomatoes. In order to have the ends snap back after sterilization when canning cold-packed tomatoes, it is necessary to hasten the work after the tomatoes are scalded. The peeling, filling and capping must be done with great rapidity in order to prevent fermentation with the

formation of even small quantities of carbonic acid gas which will prevent the ends of the cans from snapping back after the process. If there is any delay between the scalding and the processing, fermentation will begin, particularly when the thermometer is in the nineties, and of course carbonic acid gas is formed in this fermentation.

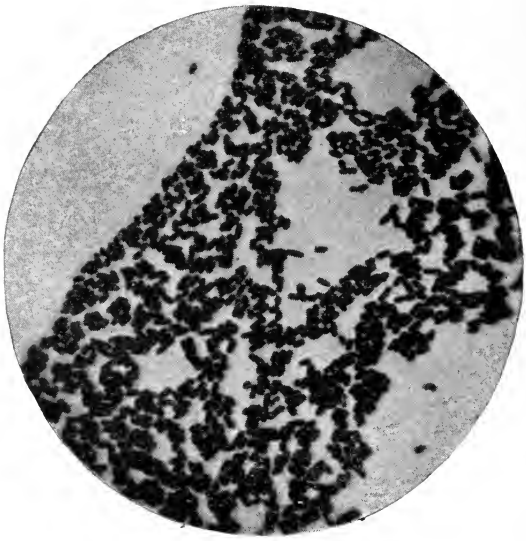


Plate 156

Photomicrograph of the vinegar bacillus. Bacillus Acidi Aceti, which was isolated from a leaky can of tomatoes. This is one of the organisms which is usually found in the "mother" of vinegar, which is called Mycoderm Aceti. Solutions containing alcohol in amounts less than 15 per cent. are fermented and the alcohol is converted into acetic acid. Stained with fuchsine and photographed through the microscope. Magnified 1,200 diameters.

ANOTHER CASE OF SPOILAGE.

We investigated the cause of the spoilage of tomatoes from the six cans expressed to the laboratory. First we examined these cans carefully for leaks and found them absolutely well sealed and no leaks in any part of them. We then sterilized the surface of the tin by using a Bunsen flame, then with a sterile awl we punched holes in the cans and took out some of the tomato juice on a sterile platinum loop and inoculated dishes and tubes containing nutrient agar, also other tubes containing sterile tomato juice. The cans contained much gas and the juice boiled out of them freely after they were punctured. We examined some of this juice under the microscope and found it full of bacteria, some of them motile, others having no distinct motion. We examined the fermented tomatoes, and found that the seeds still retained the gelatinous substance which

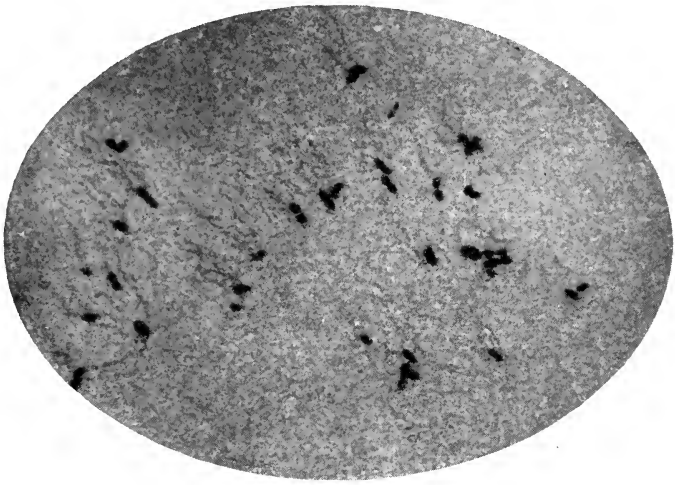


Plate 157

Photomicrograph of Bacterium Prodigiosum, a microbe which produces a beautiful red pigment, which is insoluble in water, soluble in alcohol and ether. The color is intensified by acids and turns orange-yellow by alkalis. The bacillus is motile, having numerous long flagella. It produces methylamin and ammonia and sometimes produces a gas having the odor of herring brine. Produces formic acid and carbonic acid gas. It produces proteins of a poisonous nature. (Lehmann & Neumann.) Magnified 1,200 diameters.

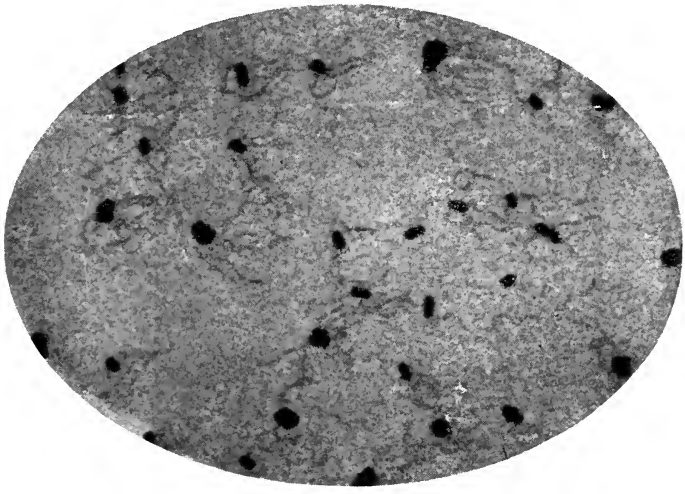


Plate 158

Photomicrograph of a colon-like bacillus which produces a light orange-colored pigment which is soluble in water. It is an actively motile bacillus and has numerous flagella. We have never before met this species in our work and will study its characteristics more thoroughly. It is not a spore-bearing organism and is easily destroyed by 180 degrees Fahrenheit moist heat. Magnified 1,200 diameters.

is found surrounding the seeds in raw tomatoes. This settled the fact that the tomatoes had received very little heating. No canned tomatoes will keep if the gelatinous substance is not cooked loose from the seeds. The heat actually required to destroy the bacteria found in tomatoes will always loosen the substance mentioned.

We frequently find that the heat has not been sufficient to destroy the life of the seeds, in which case they will grow if planted. One of the best ways of determining whether tomatoes are sucfficiently processed, is to note whether the gelatinous substance has been cooked loose from the seeds. Any process short of accomplishing this is insufficient to destroy the bacteria associated with the spoilage of tomatoes. In order to determine about what process these tomatoes had actually received, we began the work of obtaining pure cultures of the bacteria found in the cans. Four of the cans contained bacteria which would not grow in the presence of air; they were obligative anaerobes, and it was necessary for us to grow them in tubes from which the oxygen of the air was entirely excluded; this we did in the following manner: We inoculated agar containing 2 per cent glucose solidified in the form of slants in test-tubes, (nearly all anaerobic bacteria thrive better in media containing glucose), these tubes were placed in larger tubes containing a mixture of pyrogallic acid and sodium hydroxid, which rapidly absorbs the oxygen after the outer tube is hermetically sealed.

From two of the cans we could get pure cultures of aerobic bacteria. One of these was the beautiful red pigment bearing bacillus prodigiosus, sometimes found on bread, rice, and other cereals. It produces a beautiful red color. The other aerobe was also a chromogenic bacterium which produced a color of light orange. These two species were actively motile, particulary the last mentioned.

All of the bacteria isolated are species very easily destroyed by heat, none of them being able to withstand 210 degrees Fahr., and the conclusion we reached was, that these cans had not been processed long enough for even 180 degrees of heat to reach the center of the contents. Just what the conditions were, we cannot say, since we were not present, but there cannot be any question of the correctness of our conclusions because the bacteria present in the tomatoes will perish at 180 degrees Fahr., and the natural condition of the seeds is evidence that no high temperature ever reached the center of the can.

It is necessary in using any processing system, to see that the goods are subjected to the required temperature for the time necessary to accomplish sterilization, and the accomplishment of this

end is under control of the operator, whether working with the ordinary cooking vats or kettles, or with a continuous conveying system.

SWELLED TOMATOES.

We have received several samples of swelled tomatoes, the cause of which we find is due to leaky cans. The following is a sample:

When the samples of swelled tomatoes reached us several cans were burst in the seams and the contents were gone. We took a can which had no apparent leak and after incubating it examined the juice and found two species of bacteria present. One was the acetic acid bacterium and the other was a very actively motile bacillus which we were able to cultivate in pure culture only on tomato agar at first. We could not get a growth on the regular beef juice agar at first, but after two subcultures we were able to get a fine growth. This is a remarkable peculiarity of some bacteria, they become accustomed to a certain kind of food and do not readily thrive when streaked on a new substance. This is a well known characteristic of many pathogenic organisms; they will grow quite well in the body, but when planted on artificial media they grow but scantily at first, but after several subcultures they multiply very readily and to some extent lose some of their pathogenic characteristics and become saphrophytic. Another peculiarity of this tomato bacillus was, after we had made several subcultures, we were unable to get a good growth on tomato juice again, but on tomato juice which had fermented it grew quite well. This would indicate that it had nothing to do with the first fermentation, but found a suitable substance after that fermentation on which it could thrive luxuriantly.

Microscopical appearance. Large, straight rods three to six times as long as broad, with square ends, and forms chains composed of several cells. The rods are beautifully flagellated, having many of these organs of locomotion attached to the entire surface. The bacillus looks something like bacillus megatherium, but differs from it in several points, its active motility, the rods are not bent, the ends are square, and it has more flagella.

The spores of this bacillus are small and centrally located in the rods, they are very resistant to heat, and could not be destroyed by the temperatures usually given tomatoes for sterilization. As we have said, however, it will not thrive readily on unfermented tomatoes, consequently we are not usually troubled with it, unless we permit the peeled tomatoes to stand too long before they are processed, or until fermentation has started.

Plate 159

Photomicrograph of a tomato bacillus greatly resembling megatherium in size and some other characteristics, but is straight and has square ends. Gives rise to foul odor in tomatoes. It is actively motile owing to its numerous flagella, which were demonstrated by our own special method from a very young agar culture. This bacillus has many peculiarities as to its food requirements. (See text.) It forms spores and is present in tomatoes only after a previous fermentation. Magnified 1,200 diameters.

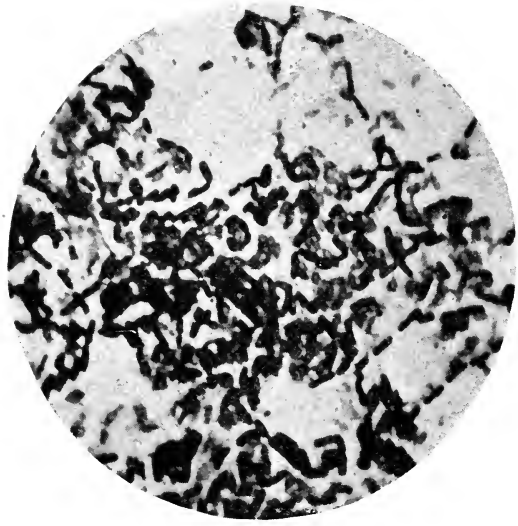


Plate 160

Photomicrograph of spores and rods of bacillus shown in Plate 159. Owing to the slime formed in the old culture, it was difficult to get a good slide preparation of the spores. The spores are quite small in proportion to the size of the rods. Stained with fuchsine. Magnified 1,000 diameters.

For a time we were unable to determine just why this organism was present in the can examined, but we finally came to the conclusion that there must be a leak in the can somewhere, so we soldered up the hole, then we attached a check valve and pumped about 25 pounds of air into the empty can. We then placed the can under water and there were several fine streams of air bubbles which came from the leaks, which were invisible to the eye.

CHAPTER XIV.

Corn

A Short Historical Sketch. The Canning of Corn. Suggestions for Canning and Processing. Cause of Sour Corn. Laboratory Work on Spoilage Cases. Spoilage Due to Poor Tin Plate. Spoilage Due to Imperfect Circulation in the Process. Bacteria Which Cause Souring of Corn. Insufficient Sterilization and Its Results. Discoloration of Corn Due to Products Elaborated by Bacteria; Other Causes. Method of Separating Sour Corn From Good. Method of Determining Cause of Spoilage, Whether Leaks or Insufficient Sterilization.

The history of corn packing dates back to the year 1839, when Isaac Winslow began his experiments. Our readers are referred to the historical sketch by Mr. F. O. Conant, of Portland, Me., in "Science and Experiment as Applied to Canning," p. 13.

Probably nothing in the canning line has given the canners more trouble than corn. It seems that it is liable to be invaded at times by extremely resistant forms of bacteria. In the early history of corn-packing, Winslow was able to sterilize his cans by simply boiling them in water for several hours. The history of his successes and failures, and not only his, but also his contemporaries, makes interesting reading. When the boiling temperature ceased to be effective, the cans were subjected to 235-240° for an hour or more, and this temperature seemed to give good satisfaction for a long time. It subsequently failed, however, and the canners had complaints of the corn turning sour.

Among the first investigators to study this problem along scientific lines were Mr. Prescott and Mr. Underwood, of the Biological Department of the Boston School of Technology. At a meeting of the Atlantic State Packers' Association held at Buffalo in February, 1898, these two gentlemen made known the results of their investigations. These papers were full of interesting and valuable information.

A great deal of the souring of corn results from two different processes, one where the souring had been accomplished prior to sterilization and the other where the same phenomenon was noticed in the cans after sterilization, and this was due to living organisms which had not been killed in the process. Strange to say, the decomposition took place without the evolution of any gas. The

sugar in the corn was converted into lactic acid, in some cases, butyric acid and valeric acid, and there was formed sulphuretted hydrogen in various amounts. It was found necessary to increase the process of corn up to 250° F. for sixty-five minutes. In some cases even this temperature has proved ineffective, but if the consistency is correct this heat will kill all spores.

As a general proposition, then, we can say that 250° for sixty-five minutes is a safe process for corn if it is not too dry. There must be enough fluid to carry the temperature from the parts nearest the tin to the center. If there is not enough fluid to do this, an impenetrable wall will form within the can, and the spores (in the center of the cans) will not be subjected to the temperature which registers on the retort, and consequently may live through any process.

Some canners are reported as being in the habit of using sulphites for bleaching purposes. This practice is extremely dangerous for several reasons. First, sulphites will attack the tin plate of poor quality and cause dark discoloration in the corn. Second, such corn has a sickly, unnatural appearance. Third, state food chemists are liable to condemn such goods as illegal, and thus bring discredit upon the whole industry. It is reported that some packers have been using saccharin for sweeting purposes instead of cane sugar. Saccharin has been declared injurious by some authorities, although I have never heard of any experiments being made to determine the truth of the statement. A favorite argument of food experts is that saccharin is a fraud, it is used as a substitute for cane sugar, and therefore is an adulterant in the eyes of the law. We cannot enter into argument, but it might be well to cease using saccharin until some definite understanding is reached on this point.

Corn, when it is delivered at the factory, should be worked up as rapidly as possible. It is the custom in some houses to pack two grades—a first and a second grade. Frequently, the whole first grade is run through while the corn intended for the second grade is piled up in great heaps. These heaps may remain long enough to have lactic decomposition set in, and sour corn will surely result, because the lactic acid formed in the corn can never be cooked out again by any sterilizing process. Corn, when delivered at the factory, contains a large number of spore-bearing bacteria of various kinds on the husks, on the silk and between the kernels of corn. When the corn is husked and run through the corn cutter these spores are thoroughly mixed in with the corn. The corn goes through the silking machines and then into the cooker and all of the fully developed bacilli are destroyed by the cooking, but the spores are not—they go into the cans and into the final process, and

that process must be about 250° for sixty-five minutes in order to insure perfect sterilization.

The calcium system offers some improvement over the retort for sterilization because the cans are somewhat agitated while going through the bath. This system is cheaper than any other and is attended with less steam and general inconvenience than the regular retort. In the following pages we will give some actual laboratory work done on spoiled corn and the results obtained, and this will be valuable to the canner.

The following is an extract of an address delivered before the canners at Columbus, O., in February, 1905.

Of all canned goods, corn seems to have given the packer more trouble than anything else—there were eighteen separate investigations of spoiled corn—and it will, no doubt, be interesting for us to draw some conclusion from the experience we have had with so many different cases of spoilage.

If you will remember, last year I stated that in nine cases out of ten the

CAUSE OF SOUR CORN

was due to the souring of the raw material before it was canned. While this statement was true at that time, it is not true today. The majority of the cans of spoiled corn investigated during this year at the laboratory, were sour on account of incomplete sterilization. There were two or three cases only of sour corn which had soured previous to the sterilizing process. Let us state the nature of this spoilage: In the first place we find that there are two distinct forms of spoilage due to insufficient sterilization.

In one, the can swells and the contents become putrid, the pressure of gas sometimes exceeding 35 to 40 pounds, to the square inch. We made a very interesting experiment to determine the pressure necessary to burst a certain make of cans, as follows: We attached the can to a steam autoclav, or steam retort, and raised the pressure gradually up to 30 pounds without bursting the can. We were afraid to raise it any higher for fear of some accident, but we are satisfied that it would have required at least 35 to 40 pounds' pressure to burst the can.

The bursting of the cans is quite a common phenomenon seen in piles of corn, so that the pressure produced by the bacteria, which are responsible for the process of decomposition, is probably more than 35 or 40 pounds. Bacteria, which produce gas in canned corn, are generally, although not always, anaerobic; that is, they will not grow in the presence of air. Most of this species are common in the soil and in decomposing organic matter. There is another class of germs which produce gas and cause the spoilage of corn; these

are aerobic; that is, they are able to grow in the presence of oxygen. Both of these varieties produce spores of great vitality.

The other form of spoilage is a souring of the contents of the can without any outward appearance of the trouble within. Sometimes these goods when opened taste remarkably well on the surface, but in the center they are putrid and the odor is abominable. This class of germs is generally aerobic, and the spores are probably more resistant to high temperatures than the gas producers.

These bacteria certainly give the canner considerable trouble, because the cans do not swell, and it is a very tedious and troublesome matter to pick out those cans which are good and those which are bad.

The following test gave excellent results in one case where it was carried out carefully: The cold cans of corn are put in the steam retort and the temperature was raised to 240 degrees, and maintained so for 65 minutes. They were then taken out of the retorts as soon as the pressure ran down, and put into cold water for five minutes. They were then piled out in rows so that both ends were visible. After three or four hours many of the ends were drawn in; these were sorted out and heated for about seven minutes at 150 degrees—all cans which did not swell in this temperature were good. Any which showed slight swelling were somewhat affected. Some of the cans were swelled on one end, while the other end had drawn in; some of these when again heated did not swell, and were good. All cans which do not swell in this second heating can be marketed as first-class goods. The balance are affected more or less and are probably a total loss.

I will explain the cause of this peculiar phenomenon. The bacteria which cause the souring and putrefaction of canned corn without swelling cans produce a substance called sulphuretted hydrogen. This is a gas which is taken up by liquids and does not affect the vacuum until it has saturated the liquid, when the surplus will cause the swelling of the can. It rarely happens, however, that it will be formed in sufficient quantities to cause the swelling, and as fast as it is generated it is usually absorbed by the fluid. When the cans are put into a retort and the temperature brought up to 250 degrees for 65 minutes the heat expands the gas and it is liberated from the fluid so that it will force both ends out and will not be absorbed again by the liquid until it has become quite cold. Usually there is enough gas left unabsorbed to destroy the vacuum and this will leave the ends puffed out somewhat even after cooling.

Nearly every packer who has experienced losses of this kind has noticed that a large number of cans are not thus affected. These good cans are scattered throughout the pile in various percentages. Now, the question comes up, why is it that a certain proportion will

spoil while the balance will be good? Why is it that the process was sufficient in one case to destroy the resistant forms of bacteria and was not sufficient in the other case? All the cans were run through the same process and were treated apparently in the same manner all through the various processes of manufacture, and yet some of them will spoil and some of them will be good.

Whenever spoilage occurs in any kind of goods this phenomenon is generally noticed, and the reason for this may be thus explained: It is generally due to variation in the consistency.

A careful study was made of the consistency of different cans of corn in the various cases of spoilage investigated. Where the corn was quite dry and very little juice was present, bacteria seemed not to have been destroyed. Corn is not easily penetrated by heat. The kernels which lie next to the tin are heated very soon after the proper temperature is registered on the retort thermometer; probably the kernels next to them are heated sufficiently; then we can imagine a sort of impenetrable wall of corn all around the can which takes up nearly all the heat and prevents it from penetrating to the center of the can. In the center of the can are numbers of spores; these spores gain entrance to the corn in the mixer and cooker; they are not destroyed in the cooker and pass through the filling machines into the cans without being harmed in the least. These spores in the center of the can, surrounded and protected as they are by an impenetrable wall of corn, withstand temperatures registered on the retort thermometer which would otherwise be sufficient to destroy all life. If there were just a sufficient amount of juice to flow in between the grains of corn and penetrate to the center, this juice would carry the heat necessary to destroy spore life; 250 degrees Fahrenheit for ten to fifteen minutes will absolutely destroy the most resistant spores known, and all that is required is to add to this time the number of minutes necessary for that temperature to register at the center of the can. I would make the suggestion, therefore, that the canners adopt a rule of adding a certain quantity of brine to each can in order to insure sufficient moisture to carry the heat to all parts of the can.

USE OF STARCH DANGEROUS.

There may be some packers who use a little starch in order to give the corn a creamy consistency. In the light of what we have said, this practice may be considered dangerous, because starch will interfere with the fluidity. Nearly every packer knows the result of delays prior to sterilization. Sour corn results from piling the husked corn in heaps, where it becomes heated. There were only two cases of sour corn from delays of this kind, and we take it for granted that the packers have become familiar with the dangers of unnecessary delays before sterilization.

SOME LABORATORY WORK ON CORN.

SOURING OF CORN.

NATIONAL CANNERS' LABORATORY,
Aspinwall, Pa.

GENTLEMEN:—We are making you an express shipment prepaid today of 1 dozen cans corn, on the bottoms of which some are marked "G" for good, and some "S" for sour.

We wish you to make an examination of these goods and let us have your report as to what you find. All these goods were processed exactly the same, viz.: 63 minutes at 250 degrees F., with hot water and steam combined, and we would like to know why it is in the condition it is.

When the samples of sour corn arrived we made culture preparations, and inoculated them with the juice from each can, taken under aseptic precautions. The culture dishes showed no growth

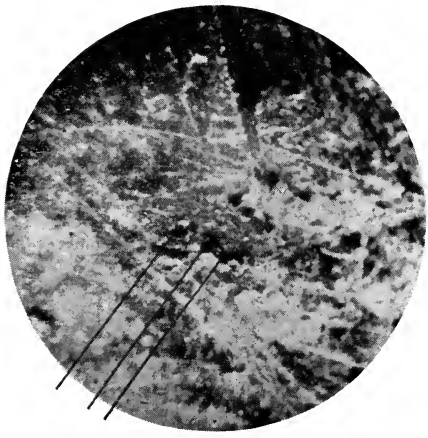


Plate 161

Defective tin from can of corn. Photomicrograph of outside surface showing three holes near the center. Magnified 200 diameters.

of bacteria, except from two cans; the balance were incubated at 98° F., until the agar dried up, but no colonies made their appearance. From two cans, however, we obtained a number of colonies and upon examination we found that some of these were micrococci, and ordinary lactic acid bacteria, which could not possibly withstand any sterilization commonly given canned corn. Even boiling temperature destroys these bacteria. Our idea was at first that the cultures were contaminations from the air, and we discarded these and prepared another set of dishes and obtained similar results. We

therefore concluded that we must have exposed the juice to the air and reasoned that the cans were contaminated by some carelessness or oversight in our manipulations. We therefore opened all the cans and examined the contents. All were sweet and good excepting the two cans from which we had obtained cultures. The juice in these cans seemed to have a watery and curdled appearance, the thin fluid presenting a faintly bluish cast by diffused light. We examined the soldering of these cans critically, looking for pin hole leaks, but none were found, so we began the search over the surface of the tin for imperfections and perforations. On the outside of the cans there were numerous rust spots having dark centers, and we found various places where the tin was perforated with exceedingly small holes. The photomicrographs show three holes,

Plate 162

Defective tin from same can as plate 161 Photomicrograph of inside surface directly opposite of plate 1, showing same three holes. Magnified 200 diameters.

one was taken from the inside surface and the other from the outside surface directly opposite from each other, under a magnification of 200 diameters.

This discovery, of course, cleared up the mysterious appearance of the non-sporulating varieties of bacteria which made their appearance in the Petri dishes. Not satisfied with this investigation, we notified the packers to forward another lot of cans, and upon receipt of these we prepared a large number (about 30) of Petri dishes, using as a medium for cultivating the bacteria, pure sweet corn juice containing 1½ per cent agar, marking each dish with the number indicated on the can from which it was taken. After twenty-four hours the dishes from several cans contained very small colonies, and after several hours more we were able to isolate the bacteria.

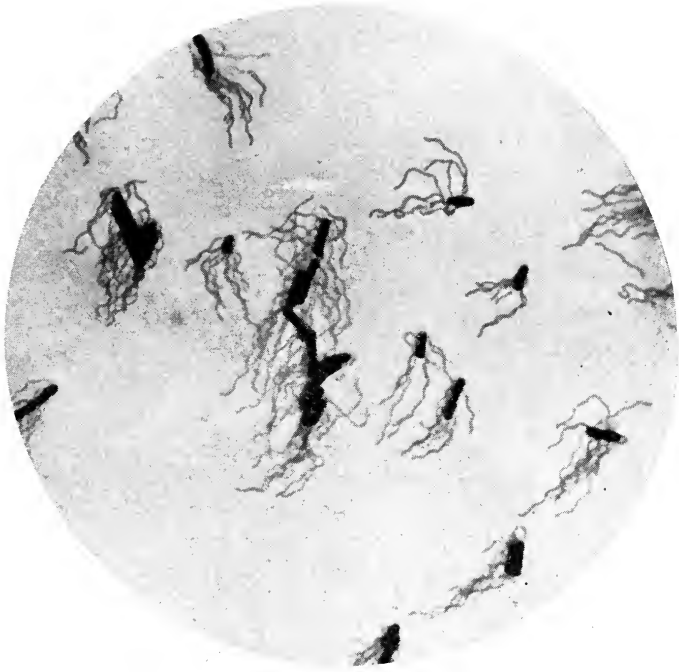


Plate 163. Bacillus Mesentericus Fuscus

Photomicrograph showing bacilli endowed with numerous flagella. This organism was isolated from a can of sour corn. It produces no gas and gives rise to spores of great vitality. Magnified 1,500 diameters.

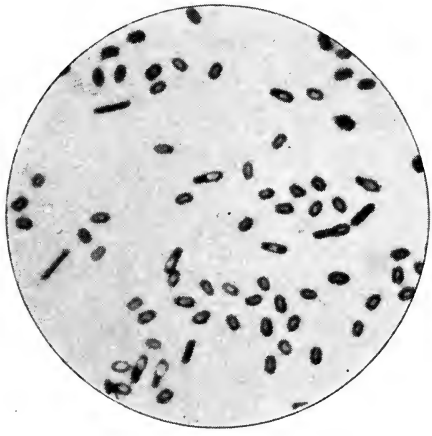


Plate 164. Bacillus Mesentericus Fuscus, showing Spores

Magnified 1,500 diameters.

One colony, first examined, was perfectly round, evenly bordered, very dark in the center, shaded down to a yellow near the edge, under a magnification of 60 diameters by transmitted light.

The natural size was about one millimeter in diameter, and had a lustrous bluish cast with a dark spot in the center. This proved to be the lactic acid bacillus and numerous similar colonies afterwards made their appearance. We have shown the illustration (of lactic acid bacteria) see Plate 39. This was from a perforated can.

Another colony was examined which resembled the above very closely except that it had a crumby appearance. It looked as if it had broken glass or sand sprinkled over the surface. We made a streak culture of this organism and found that it was a motile spore bearing bacillus, which produced no gas. It grew rapidly over the surface and seemed to grow downward into the agar as well as over the surface, the center of the growth being covered with small wrinkled folds, while the extending layer was very thin and almost transparent. The organism grew well both as an aerobe and as an anaerobe, but in the last named condition seemed to produce more acid, which was something like phosphoric acid, very sour. The growth on corn juice was rapid with no gas, and the juice seemed to present the same curdled appearance noticed on opening the cans of sour corn.

The rods begin to form spores after the second day and when examined in the living state a degeneration of the cell may be observed, the protoplasm becoming less homogeneous, and sporangic granules are seen as is the case during plasmolysis. After a time the granules seem to collect and a bright shining spot appears near one end of the rod, and this spot seems to take on a definite shape; it is the spore forming within the cell. Our photomicrograph shows some of these rods which have the spores within. There are a number of rods which are barren, that is to say, they will not produce spores; such rods are met with in nearly all cultures. The membranes of these spores are quite thick and are able therefore to withstand high temperatures, or other unfavorable conditions, and afterwards develop into vegetating rods when conditions of environment are favorable. In order to give the packers some idea of the size of such spores, it would take about 25,000 of them placed side by side to measure one inch; it would take 200 of them, placed end to end, to reach across a hair. An ordinary pin hole leak in a tin can is large enough to admit 50,000 of these spores at one time if that many could be collected and crowded together. The photomicrographs of the various species are magnified from 1,000 to 1,500 diameters, which is a real magnification of from one to two and a half million times, so that our readers can appreciate the

extreme delicacy of the photographers's work, which must be done through the microscope, everything being absolutely quiet and the brightest radiant possible. The can from which bacillus Mesentericus Fuscus was taken was not a leak, but the sterilization was incomplete.

In another can of sour corn we found another kind of organism associated with the bacillus just described. We designated this as bacillus Liodermos.

After twenty-four hours, fine water points appeared on the surface of the culture medium, and as they grew older these colonies took on a reddish yellow color at a magnification of 60 diameters by

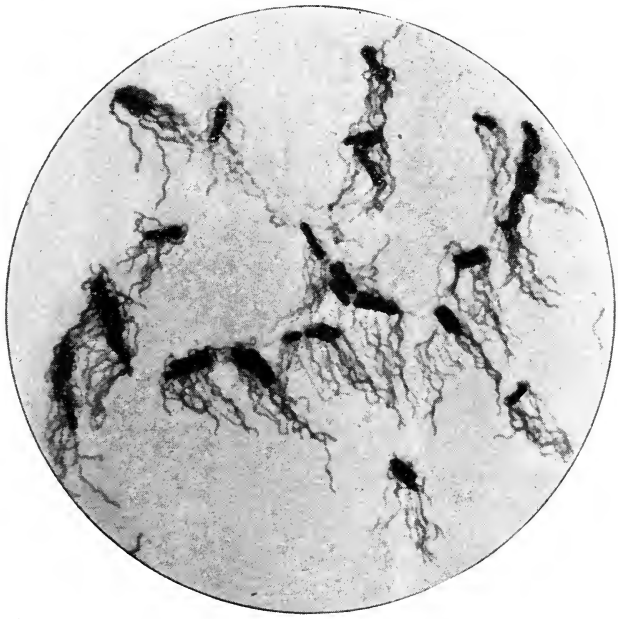


Plate 165. Bacillus Liodermos

Photomicrograph of bacilli showing their numerous flagella which are somewhat matted together. Vegetating rods obtained from a culture on Agar incubated at 98 degrees Fahrenheit for eight hours. Flagella stained by our own method. This organism was isolated from a can of sour corn. It produces no gas, forms butyric acid and H2 S. Magnified 1,500 diameters.

transmitted light. Naturally the colonies are about 2 m. m. in diameter and round, somewhat elevated, but when magnified slight growths can be seen extending outward from the periphery. The agar streak culture is a moistly shining, dirty layer, with a thin, punctuated, transparent growth pushing ahead of the older streak. This layer rapidly extends to the walls of the dish even up the sides for a short distance. The bacilli are about 3 to 6 μ long, having numerous hair-like organs of locomotion. They form chains

and have a tendency to collect in bunches, being matted together, held, no doubt, by interlaced flagella. Our photomicrograph shows them thus connected. This bacillus produces butyric acid and sulphuretted hydrogen, but no gas. When we tasted the juice of this can it was somewhat disagreeable, having not only a sour taste but a flavor not at all pleasant.

This bacillus forms spores which are located at the center of the rods, and are extremely resistant to high temperature. We have reserved a culture of them in the laboratory for further study.

One can contained, with others, a bacillus which produced a bitter principle not unlike that of raw peas. The juice of this can

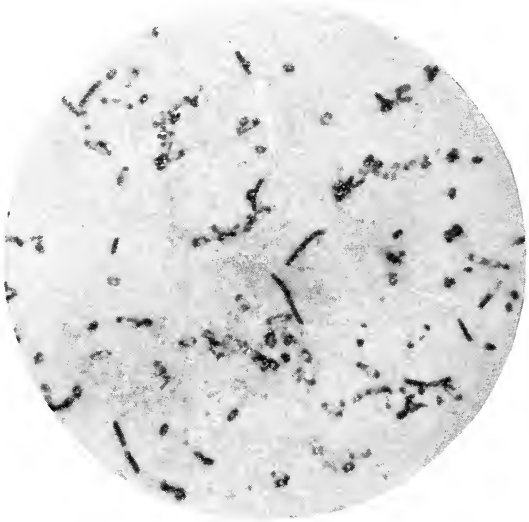


Plate 166. Bacillus Liodermos

Photomicrograph showing rods containing median spores, barren rod and free spores. This preparation was made from a culture on Agar four days old, and stained with carbol fuchsine. These spores are the seed forms of the bacilli shown in Plate 165. They are very resistant to heat, being able to withstand boiling for several hours. Magnified 1,500 diameters.

was different in flavor from any of the others. This was marked Can No. 1, and when the colonies began to grow we noted other varieties previously examined, also this one, which is designated as Bacillus Bittergenus.

The colonies were all deeply imbedded in the agar, none at all appearing on the surface, which indicates that it favors an anaerobic condition. The streak also had a tendency to grow downward into the medium. The deep colonies were opaque, granular and brown. When planted in sweet corn juice a bitter flavor was imparted. The spores are medium.

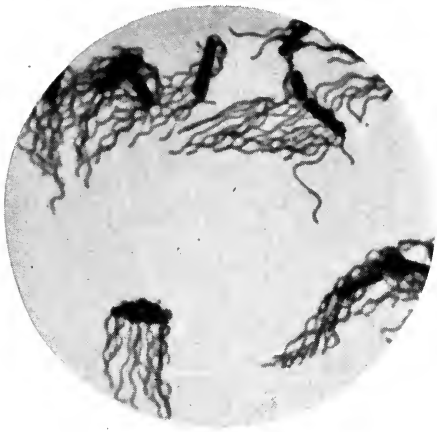


Plate 167

Photomicrograph of Bacillus Bittergenus, a very actively motile bacillus isolated from a can of bitter, sour corn. It has numerous flagella and stains well by our laboratory method. It is an anaerobe facultative aerobe and imparts a slightly bitter flavor to corn. Magnified 1,500 diameters.

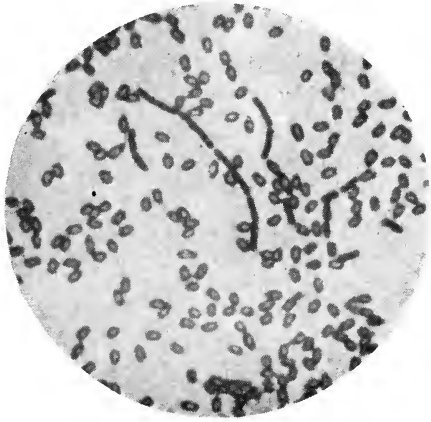


Plate 168

Photomicrograph of the spore forms of Bacillus Bittergenus. The spores are median and quite large in comparison with the breadth of the rods. This specimen was stained with carbol fuchsine from an Agar culture and tested for vitality. It resists boiling for hours. Magnified 1,500 diameters.

The sample of salt proved to be as good as any ordinary table salt, and showed no acid reaction, being neutral.

The sugar crystals proved to be saccharin, giving the violet reaction by the ferric chlorid test, after being converted into salicylic acid.

CONCLUSIONS.

The cans used for this lot of corn had a very inferior coating of tin, and we would recommend a better quality of tin plate for packing corn.

The process of 250° F. for 63 minutes would seem to be sufficient if the circulation were all right. We do not regard a steam and water process as reliable as dry steam, for the reason that water has not as good circulation as dry steam. The same process with dry steam and free exhaust will undoubtedly sterilize the corn perfectly.

This process will discolor corn slightly unless the cans are chilled with cold water. Our experience has been that the best way to accomplish this is to run cold water into the retorts through the lid before opening them, when the temperature falls to about 220° F.

We would recommend that the use of saccharin be discontinued, because it is illegal in many states, and canned goods or any other food containing this sweetener, are liable to be analyzed and condemned by the authorities.

MR. E. W. DUCKWALL,
Aspinwall, Pa.

DEAR SIR:—We have been surprised to learn that some of our corn has turned sour. Only one or two cans in a case have been discovered so far and they are among the solid pack of 1903. We are sending you by express prepaid several cans which may be sour. We would like to have you make a bacteriological examination of these, and would be pleased to hear the results. We have been processing at 245° F. for sixty-five minutes, but this is probably too low. Would you advise us to increase it to 250° F., as you suggest in your writings? Awaiting your reply, we remain,

Yours very truly,

_____.

Only two cans were found to be sour in the case of goods received, and we made plate cultures of the juice by streaking a number of Petri dishes containing nutrient corn juice agar. Within thirty-six hours we had a number of colonies, many of which were the same. We found only two colonies which showed any marked difference. These we transplanted into bouillon, and pellicles formed rapidly on the surface, one being much more wrinkled than the other, growing fast to the walls of the tube, and not easily precipitated by shaking.

From the bouillon culture we streaked new culture plates, and obtained a rapidly spreading growth in eight hours, which was composed of very motile bacteria. From this growth we obtained a slide preparation and stained it for flagella.

The central portion grew much elevated with folded white wrinkles; from these we were able to get a specimen of the spores,

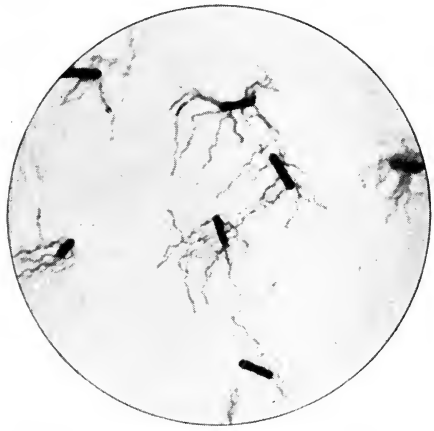


Plate 169. Bacillus Mesentericus Frumenti

Photomicrograph of a Corn Bacillus found in a can of sour corn, which had been sterilized at 245 degrees Fahrenheit for 65 minutes. It produces much acid no gas and curdles the corn milk. It is actively motile, propelling itself by means of numerous flagella growing out from the cell in all directions. This is from a very young growth on corn juice agar, stained by our own method, mounted in Xylol Balsam. Magnified 1,000 diameters.

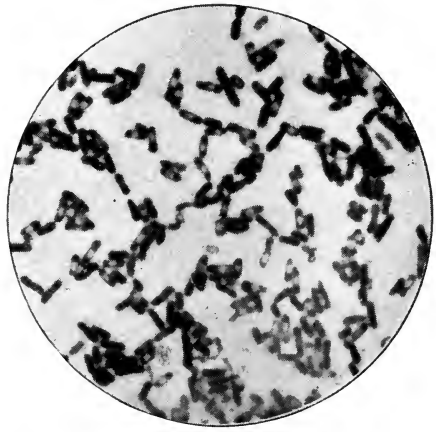


Plate 170. Bacillus Mesentericus Frumenti

Photomicrograph of the spores of the Corn Bacillus, shown in plate 169. These spores are centrally located in the rods, and after being set free are able to resist a temperature of 245 degrees Fahrenheit for 65 minutes when growing in cans of corn. Stained with carbol fuchsine, mounted in Xylol Balsam, photographed with acetylene radiant under a 1-12 homogeneous oil immersion lens, giving a magnification of 1,000 diameters.

which resembled other varieties previously examined. The spores had very thick walls and were thus well protected against heat or other unfavorable conditions. This organism seemed to curdle the corn juice giving it a greyish color, and the water seemed to separate from the juice quite freely. It was possible to detect this watery condition by shaking the along with one not so affected.

The other bacillus corresponded to one previously described (see Plate 165).

SPOILED CORN.

The following letter explains itself:

MR. E. W. DUCKWALL,
Aspinwall, Pa.

DEAR SIR:—We are expressing you today, prepaid, four cans, two bulged, one good, and one empty, for examination. Please advise us wherein the trouble lies. Is it a lack of sterilization, faulty cans or our water supply? We use a standard formula, cooking one hour and twenty minutes at 240°. Our water supply comes from a driven well 178 feet deep. Do you consider the good can safe?

Very truly yours,

_____.

The four cans reached the laboratory, but the contents of one had been lost on the way. We punctured the other swells with a sterilized awl, and after the escape of fermenting corn juice and malodorous gases, we inoculated several Petri dishes and bouillon tubes with some of the juice taken from the can, under as nearly aseptic conditions as possible. We had a fine growth of bacteria in all of the tubes and dishes. After diluting the bouillon we streaked several Petri dishes, and obtained colonies sufficiently separated for isolation. One of these organisms was described under plate 169. The bouillon culture formed a pellicle on the surface and this became very much wrinkled. The plate culture spread rapidly, a thin, transparent growth of bacteria extending in all directions from the line of inoculation. This organism produced no gas in any of the cultures made, and when transplanted into cans of corn caused them to turn sour, without the formation of gas or swelling of the can. It forms spores, centrally located in the rods, and when these are set free they become extremely resistant to high temperatures and are able to withstand 245 degrees F. for 65 minutes, and in this case withstood a temperature of 240 degrees for 80 minutes. This was the process mentioned by the packer in his letter.

The other bacillus formed gas quite freely, and sulphuretted hydrogen was also formed, and the bouillon culture gave the indol reactions. The foul odor noticed when we opened the can was due to these two products. The bacillus is a spore bearing, actively motile organisms and formed a pellicle on the surface of the bouillon, and caused the fermentation of grape sugar bouillon and corn juice. It is endowed with numerous flagella, which grow out from the entire surface of the cells, and these are the cause of its active motility. The colonies on agar are round and greyish white, at first lustrous, then becoming more wrinkled and folded. The periphery

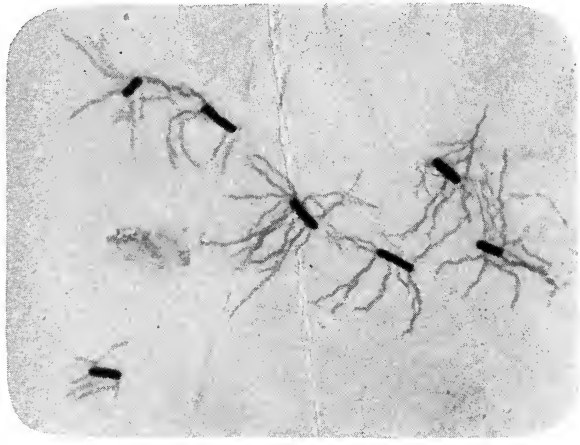


Plate 171. Bacillus of Malodorous Corn

Photomicrograph of a bacillus isolated from a can of spoiled corn which had been processed for 80 minutes at 240 degrees Fahrenheit. This organism actively motile, having very numerous flagella stained by our special method. This specimen was taken from a very young growth of pure culture on Agar. Magnified 1,200 diameters.

is indented or tufted; the same general characteristics are demonstrated in the streak culture on the surface of nutrient agar in Petri dishes. The spores are located nearer one end of the rod, and when set free are very resistent to high temperatures, as was evident from their having passed through a process of 240 degrees F. for 80 minutes. The cans inoculated with the spores of this organism were perfectly sterilized when given a process of 250 degrees F. for 65 minutes.

In sterilizing corn we believe that 250 degrees F. should be used in preference to the lower temperature. It requires about 50 to 55 minutes for this temperature to reach the center of a No. 2 can, and it requires about 10 minutes' exposure to this temperature to insure devitalization of the spores of such bacteria as are shown in the accompanying plates. It is barely possible that this time

could be cut down a few minutes if the cans are agitated during sterilization, but corn is almost impervious to heat, and those kernels which are nearest the tin are sterilized in far less time than those at the center, so that agitation would have a tendency to bring the corn in the center nearer the in at times. A temperature of 250 degrees does darken the color a trifle, but if the proper cooling process is employed, the color will be very good, and while not as white as corn bleached with sulphites, is good enough for any market. Some of the cans sent to the laboratory for inspection have had very good color after this process. The good can appears to be all right; up to this time it has not swelled in a temperature of 98 degrees F.

DISCOLORATION OF CORN.

NATIONAL CANNERS' LABORATORY,
Aspinwall, Pa.

GENTLEMEN:—We are sending you via express today four cans corn. We wish you to analyze same and kindly write us as soon as possible cause for their being curdled like and black in cap end of can. Corn looks good except on capped end. Our whole pack seems to be in same shape. We have taken these cans from four different weeks' pack.

Yours very truly,

_____.

When the cans arrived we placed all excepting one in the incubator for a week in order to get a growth of any bacteria which might be present. After that time we removed them and streaked a number of Petri dishes containing a nutrient agar preparation. We examined the juice carefully, but could find no trace of bacteria at that time. We naturally thought that the cans contained no bacteria, because we could not see any under the microscope, but we obtained a free growth in the Petri dishes within twenty-four hours. We then examined the cans again and found that in two of them the bacteria had developed wonderfully. We did not get any growth from one can, either in the can or in the dishes, so this can was sterile. These bacteria were strictly aerobic, that is, they were able to grow only in the presence of air, and this accounted for our failure to make them multiply in the cans during the first incubation. We endeavored to grow them in the anaerobic culture apparatus, but were unsuccessful, and this proved that they were strict aerobes. There were two different species, one in can marked No. 1, and

another in can No. 3, and both were motile and spore bearing. We introduced the spores into some cans of good corn we have in the laboratory, then processed them; within a week we had the same discoloration seen in the originals. This would indicate that the bacteria were able to grow as long as there was any oxygen in the can, and after that they would form spores and the rods would dissolve,

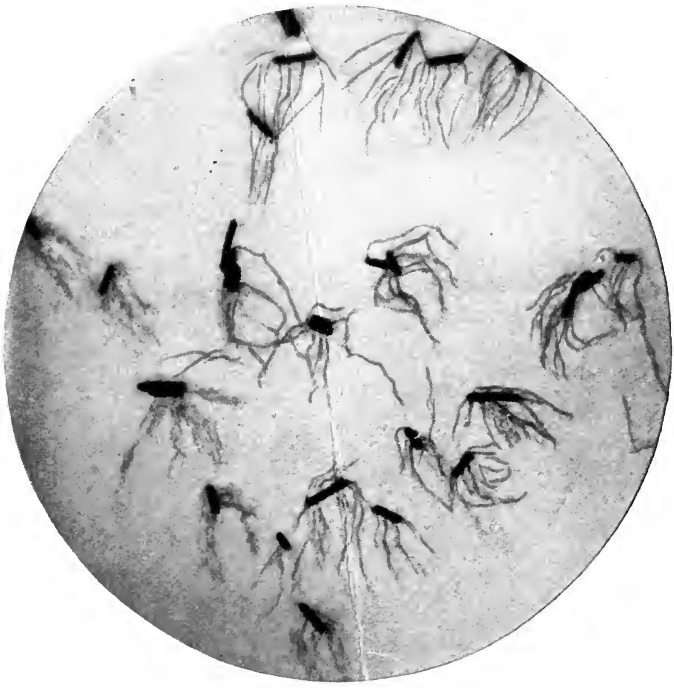


Plate 172

Photomicrograph of a beautifully flagellated bacillus which greatly resembles Bacillus Mesentericus Vulgatus in many respects, but is an obligative aerobe. Isolated from a can of corn, which showed dark discoloration, due to the action of sulphuretted hydrogen on the metal of the can. Slide preparation made from a six hours' growth on agar. The numerous flagella are stained by our own special method, and photographed through the microscope through a 1-12 homogeneous oil immersion objective and No. 6 compensating eyepiece, using acetylene radiant. Magnified 1,200 diameters.

leaving only free spores which we were unable to detect positively in the juice under the microscope.

The bacillus found in can No. 1 was beautifully flageliated, which gave it very active motility. It is strictly aerobic and differs from any bacillus thus far isolated by us. In appearance it greatly resembles bacillus mesentericus vulgatus. It forms long chains and some of these would be motile and flagellated. After a time spores would form near one end of the rods and the rods would dissolve rapidly, leaving the spores free.

Plate culture on agar.—Very rapid growth with scalloped border. A thin almost transparent film of young motile bacteria extended in all directions and soon reaches the walls of the dish. From this almost invisible growth fine preparation may be obtained for the demonstration of the flagella. After the growth becomes quite visible it is slimy, and in this resembles the potato bacillus. It forms a pellicle on the surface of bouillon, but does not produce any gas. It forms a small amount of sulphuretted hydrogen, which is determined as follows: The agar is colored a light yellow with ferritatrate made alkaline with sodium carbonate; the bacteria cause

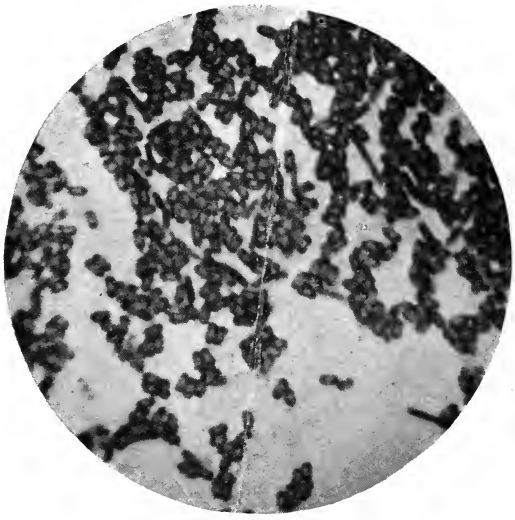


Plate 173

Photomicrograph of the thick walled spores of the bacillus shown in plate 172. The spores are the seed forms and are exceedingly resistant to heat and other unfavorable conditions. These are the forms of the bacillus, when forced into a resting state. In a favorable place they will again form the bacilli, providing air is present. This photograph was taken from a slide preparation of a four days' growth of a pure culture on agar. Magnified 1,500 diameters.

this to turn black if sulphuretted hydrogen is produced. It is this substance which gives the corn the dark discoloration seen frequently and was the direct cause in the case before us.

The spores of this organism are very resistant to heat, because they are thick membraned. A close study of the plate shows this characteristic. Nearly every rod forms spores and when we stained an old culture we rarely found any rods at all, they all having gone to spores.

This organism grows only in the presence of air, and when forced into an anaerobic condition forms spores and goes into a resting state. For this reason it will not continue to grow in the sealed

cans after the supply of oxygen is exhausted, consequently will not cause very great changes.

The bacillus isolated from the can marked No. 3, was different in its manner of growth. When streaked on agar it grew only along the line of inoculation and did not send out the invisible film seen in the other culture. On ordinary agar we had difficulty in getting a confluent growth; it seemed to form colonies and resembled Hueppe's bacillus butyricus in this respect. It did not form any slime, but gave rise to spores rapidly. These spores are centrally

Plate 174

Photomicrograph of a very active bacillus greatly resembling Hueppe's Bacillus butyricus in its manner of growth and chemical products, but is an obligative aerobe. Culture obtained from a can of discolored corn. It produces no gas but forms small amounts of sulphuretted hydrogen. It also produces butyric acid. Slide preparation was made from a very young growth on agar and the numerous flagella were demonstrated by our special method and then photographed through the microscope using acetylene radient. Magnified 1,200 diameters.

located in the rods as a rule, although some of them were quite near the ends of the rods. There were a great many barren rods in the cultures we made, and many rods which did not form spores at all, as shown by the plate. The spores are quite small, and the membrane does not seem to be as thick as that of the other species. This organism is also strictly aerobic, and cannot grow where oxygen is excluded. We went through the same technique as described

previously and learned that sulphuretted hydrogen was produced, but only in small amounts. It produced no indol or other foul product and formed no gas. Like the other species it developed and grew as long as there was any oxygen in the can and then went into a resting state.

These are the first strictly aerobic bacteria we have ever isolated from canned corn, all the others have been facultative anaerobes; that is, they were able to grow in the presence or absence of oxygen. That class of bacteria would of course continue to grow

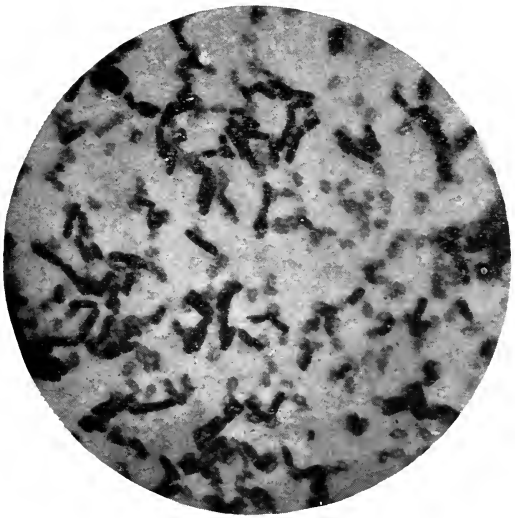


Plate 175

Photomicrograph of the spores and barren rods of bacillus shown in plate 174. The spores are smaller than those of the first bacillus obtained from can No. 1, and the walls of the spores are more delicate. They are able, however, to withstand much heat. From four days agar culture, stained with carbol fuchsin. Magnified 1,500 diameters.

and completely ruin the goods. While these have interfered somewhat with the appearance, they cannot proceed and the goods will not therefore deteriorate any further.

We would recommend a process of 250 degrees F. for 65 minutes, which will be sufficient, we believe, to insure perfect sterilization.

SOUR CORN.

A packer called at the laboratory and stated that he had been losing quite heavily on one particular brand of his corn on account of sourness. He stated that there were other canners in his immediate vicinity who were also having similar trouble.

This packer puts up a special grade of corn and his loss was confined to this grade. He said that he had not lost any of the regular goods from "sours." The special grade he had put up in the usual manner and used granulated sugar as a sweetener, where formerly he had used saccharin. He said that when he opened some of the cans they were quite sour and some had a putrefying odor. None of the cans showed any signs of swelling. He processed these goods 55 minutes at 240 degrees Fahr. and allowed about 20 minutes' time for heating up to that temperature. He was very anxious to sort out the bad cans so that he might dispose of all that were good. He had tried in various ways to do this, but none of them proved reliable, so he came to the laboratory for advice.

We requested him to send us a case of this corn for bacteriological examination. This he did. We advised him in the meantime to heat his cans in boiling water until they all swelled. This we thought could be done in about 20 to 30 minutes, and our idea was to chill the cans quickly with cold water and all whose ends snapped back within a few minutes were to be sorted out as good cans and all which failed to snap back were to be considered bad cans. He acted on our advice and reported that among the cans whose ends snapped back quickly, he found quite a number which were sour, and among those which he had set aside as bad, he found quite a number which showed no evidence of sourness, then he stated that he was opening the cans now, and was tasting them, but he had taken a large number of cans which had been tasted, recapped them and then put them into the retorts and given them a process of 250 degrees for 65 minutes. He stated that on opening some of these cans he found that they were very much discolored by the extra amount of cooking and also that quite a number had developed sourness in the process. This mystified him very much, but I told him that the sourness had started in the center of the cans and had probably not extended to the surface, therefore it could not be detected by those who had tasted the corn before the cans were reprocessed. The processing thoroughly mixed the acid so that it was easily detected afterwards.

I told him that where corn soured on account of the bacteria developing in the cans after incomplete sterilization, it usually started in the center of the cans, because at that point the heat had not been sufficient to destroy them. During the mixing of the corn, prior to the filling, the spores of the bacteria peculiar to corn are mixed in with the mass so that there are many in the center of the cans. Some spores are destroyed at even moderate temperatures, but others will survive cooking for a long time, and unless the temperature used in sterilization is sufficiently high and prolonged to reach the resistant spore forms in the center of the cans

they will afterwards develop and the sourness will begin at the point where the bacteria began to grow.

This cleared the matter up for him and he decided to subject all of his cans to a high temperature for a sufficient time to heat them through to the center, so he used a temperature of 240 degrees Fahr. for 65 minutes and this heat thoroughly penetrated the cans so that when they were removed both ends bulged out. The color of these goods was very fair considering the amount of cooking they had received, in fact, it was almost impossible to detect any difference in the color from that of the original.

The question now arose in the packer's mind whether this second cooking would be sufficient to prevent bacterial action in those cans which he had sorted out as good; also in those cans which he had sorted out and styled "push-backs." In order to determine this point he sent some of the latter into the laboratory. We inoculated Petri dishes and bouillon with some of the juice, and after thirty-six hours we failed to get any growth. It would seem therefore that the second cooking had completely sterilized the cans. This was probably due to the fact that there were no spore forms present, all having developed into full-grown bacteria or vegetating forms, which are easily destroyed even at 180 degrees Fahr.

This packer said that about 40 per cent of the cans had soured, and he was very much at a loss to know why that per cent had soured and the other 60 per cent were apparently good. We opened quite a number of cans in his presence and examined the consistency. We found that the consistency varied considerably. Some cans were very solid, having little fluid; others were quite moist, so that the fluid flowed back and forth between the particles of corn; and there were still others whose consistency varied between these two extremes. It is quite reasonable to suppose that the solid-packed corn would be less penetrable to heat than that which was more moist, but in the first place the temperature of 240 degrees Fahr. for 55 minutes would not be a safe process for corn, and it is surprising that it did not all sour. We are of the opinion that this would have been the result finally, but owing to the temperature of the corn room being quite low, the putrefactive action was necessarily slow. Then again, the process given might have been sufficient in some cases where the spores were of less resistant character. The process for corn should be 250 degrees Fahr. for 65 minutes, not counting the time required to reach that degree of heat. By the calcium system, time for raising this temperature would have to be allowed, so that the total time for carrying cans through the calcium solution would be about 70 to 75 minutes.

The following method of testing to separate sour from sweet corn has been used with much success by some packers, and while

we are not called upon to guarantee its infallability, we have added an explanation of points which would seem to indicate its reasonableness. We should, however, be pleased to receive from any of our packer friends any data based on their experiences which would tend to throw any further light on this general subject.

TEST FOR SEPARATING SOUR FROM SWEET CORN.

Corn to be taken cold and put in steam kettles. Temperature to be run up in twenty minutes to 240 degrees; hold for sixty-five minutes at that point. Take out quickly as possible and put in cold water for five minutes.

Take crates in warehouse, and by hand, lay cans on floor in rows carefully, so each end of can is exposed to view. After standing in warehouse for four hours (temperature in warehouse to be 50 degrees), go over cans and pick out all that have collapsed on both ends. Quickly run them through the hot tank at 148 degrees at feed end and not over 151 degrees at outlet of tank. Speed of cans through tanks, seven minutes.

Such cans as pass through the hot tank undeveloped or not swelled to be set aside and called No. 1 collapse. Such cans as develop or swell when passing through hot tank to be set aside and called No. 2 collapse.

Then go over balances of cans in warehouse immediately, and all cans that have swelled on one end only, or collapse with the pressure of the finger, put all such cans through the hot tank, same temperature and speed as collapses. All cans that pass through undeveloped to be kept separate and called No. 1 push backs, and all cans that develop or swell to be set aside and called No. 2 push backs.

After this second overhauling, all cans that remain on warehouse floor swelled at both ends to be set aside as spoiled and of no value.

All cans known as No. 1 collapses to be considered first quality, and all cans known as No. 1 push backs, to be considered first quality. All cans from No. 2 collapses, to be considered second grade corn and salable.

No. 1 collapses, No. 1 push backs and No. 2 collapses, to be cooled immediately after coming out of hot tank, by laying on platform outside of building; should lay sufficient time to become thoroughly cold, not frozen.

Greatest care possible must be exercised in the handling of the cans so as not to prematurely do anything that will cause them to collapse on either end; the collapsing must be natural, with the exception that on the second over-hauling, such cans as are collapsed

on one end only, to be forced in; that is, such cans as will yield to the slight pressure of the fingers.

Referring to the above mentioned system of sorting out sour corn from the good, we will explain how this may be judged as a reasonable method. In Plate 176 we have reproduced the bacteria which had caused the sour corn. These belong to a certain class of microbes which do not form gas; they attack the carbohydrates, principally the sugar of the corn, converting it into lactic acid, and also forming other complex substances such as indol, and sulphuretted hydrogen which is taken up by the fluid and does not volatil-

Plate 176

Photomicrograph of the corn bacillus which produces no gas; it breaks up the sugar into lactic acid, butyric acid and sulphuretted hydrogen, consequently will cause "sour corn" without swelling the cans. This organism differs from all classified bacteria in several respects, although resembling Mesentericus in the heat resisting power of its spores. It is a facultative anaerobe, actively motile. Flagella are demonstrated by our own method. Magnified 1,500 diameters.

ize into gas except under the influence of heat. These chemical changes may take place in a vacuum and no gas is produced, and there is no evidence whatever from the external appearance of the can of any such changes. When the packer heated his cans so that the high temperature penetrated to the center, the hydrogen sulphdie was driven into the form of a gas, and owing to this characteristic property, the bad cans were quite easily sorted out while the good cans which contained none of the (H_2S) soon snapped back to their normal condition.

During our conversation the packer asked me if it was not possible that the granulated sugar he had used in this corn, might have had something to do with the souring. He stated that he had

formerly used saccharin or "sugar crystals," as they are called, and he had never had any such loss. He stated that it had been represented to him that sugar would cause the souring of peas and corn, and he also stated that a certain salesman of chemicals had advised him never to use granulated sugar, or if he decided to use sugar at all, he ought to use "Franklin A" or Confectioners' "A;" that granulated sugar contained impurities which might cause the souring of his good. All such talk as this is mere theory. There is nothing in the facts to warrant such statements. The chemical reports on granulated sugar show that it runs from 98 to 100 per cent pure, and a small amount of impurity would have nothing whatever to do with the spoilage of such goods as corn or peas.

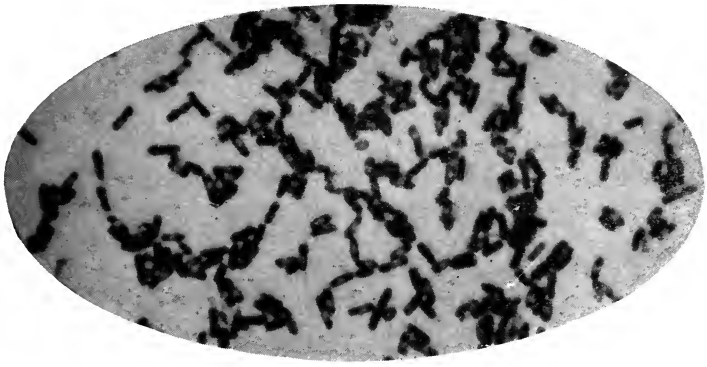


Plate 177

Photomicrograph of the spore-bearing rods of the corn bacillus shown in preceding plate. The rods show the spores located in the center. The spores are extremely resistant to high temperatures. The bacilli form chains of several rods. This photomicrograph was taken from a 48-hour growth on agar, slightly stained with carbol fuchsine, mounted and photographed through a microscope using Spencer 1-12 oil immersion objective and acetylene radiant. Magnified 1,500 diameters.

There are no extremely resistant forms of bacteria identified with the fermentation of sugar. Molds and yeasts will cause a fermentation of granulated sugar when combined with fruit juices, fruit pulps and tomato products, but these forms of life are easily destroyed at even boiling temperature. Granulated sugar is in itself a preservative, and could not increase the risk of souring in goods which must receive as high a temperature as that which is given sugar corn for complete sterilization. The cause of spoilage in canned corn after sterilization, is always traceable to spore-bearing bacteria which are present in the corn itself before canning. The sugar could not have any influence one way or the other. It has been claimed that saccharin was a preservative and for that reason was preferable to granulated sugar, because it exercised antiseptic power on the bacteria, but this is not the case simply be-

cause saccharin is used only in very limited quantities, quantities so small that it could not have any antiseptic influence.

Preservatives are valuable only when used in sufficient amounts to prevent the multiplication of bacteria; when used in small amounts they act rather as a stimulant, and are used sometimes in our culture media to stimulate the growth of certain species of bacteria. Salicylic acid is frequently used in the isolation of the typhoid bacillus which grows readily when only a small amount of this chemical is employed. Saccharin even in very strong solutions has very little antiseptic power, and in the amount used for sweetening corn and peas its influence would be rather stimulative than antiseptic.

Speaking of saccharin, we recall the heated controversy which took place at the International Food Congress in St. Louis between those who had investigated its action pharmacologically, and the Food Commissioners. The Food Commissioners looked upon saccharin as a substitute for sugar, and they claim that it has no food value, that it passes through the body in an unchanged condition, and that if used for a long time it is apt to cause nephritis. That it does pass though the body in an unchanged condition, and that it is not a food, we cannot deny. As to its effect upon the human body, we cannot dispute their claims, because we have never made any experiments to determine the correctness of these assertions. We would advise packers, if we might be permitted to make a suggestion, that they be guarded in their use of saccharin this coming season, because the food commissioners are liable to enter suits against any grocers who sell canned goods containing this artificial sweetener, and the packers will have to come forward then and defend their customers.

We are also under the impression, though we cannot state it as a positive fact, that saccharin would oxydize into salicylic acid in some cases where high temperatures are used to accomplish sterilization of canned goods. It is not a very difficult matter to determine the presence of saccharin in canned corn and peas, the chemical technique being similar to that used for determining the presence of salicylic acid, namely, a chloroform or ether extract is made and the residue from this is converted into salicylic acid by subjecting it to a high temperature, 250 degrees C., with sodium hydroxide. The sodium salicylate thus formed is easily detected by the ferric chloride test and in order to confirm this the residue from another extraction is heated with resorcin and a few drops of sulphuric acid in a test tube till it begins to swell up. Several times heating and then neutralizing with sodium hydroxide will give a red-green fluorescence where saccharin is present.

ANOTHER CASE.

We were informed that one packer claimed to have a great deal of sour corn after using a process of 250 degrees Fahr. for 70 minutes, but this does not seem likely to me. If such were the case, his corn must have been entirely too dry or solid-packed, and this is a very important matter. It must not be overlooked that sour corn is not always due to imperfect sterilization. We all know that corn will sour if allowed to remain standing in piles before canning. I was told of the condition in some of the factories which had considerable sour corn. It was something like this: They were sorting their corn after it was husked, into two grades, a No. 1 and No. 2. The No. 1 grade was run through the cutters, cooked, filled, capped and processed promptly, while the No. 2 was carried off to one side and the husked ears were piled up in great heaps. This remained in this condition until after completing the run on No. 1; then the No. 2 was run through the machine and canned. A great many "sours" developed in this No. 2, and it is no wonder, because the lactic acid bacteria would develop readily in the center of such piles and when lactic acid is formed it cannot be eliminated by any cooking process.

A GAS PRODUCING ORGANISM IN CANNED CORN.

We examined a can of corn which had received about half the process usually given corn. Our idea was to isolate all the bacteria that usually infest corn, and we expected to find several different varieties, but only one species developed, which was an anaerobe. This germ produced great quantities of gas and the can swelled up until it was nearly ready to burst when we punctured it and made plate cultures of the bacteria. While the germ was an anaerobe we could get a small growth when cultivated in the presence of air but it grew better when all oxygen was excluded. The gas produced by this organism was sulphuretted hydrogen, which we detected by the sodium ferri-tartrate test. We also found that this germ produced phenol, which probably accounted for the fact that no other organisms were present, the phenol having acted as an antiseptic. This organism produces spores rapidly in the incubator at 98 degrees Fahr., and while these spores are heat-resisting, they are not to be compared with those of the bacteria which produce no gas.

One peculiarity of this germ has induced us to mention the experiment in this issue, namely, its production of phenol; and there are quite a number of other bacteria which produce the same substance, and goods in which they are thriving will respond to the ferric chloride test for salicylic acid. We do not believe any packers ever use salicylic acid in canned corn, but we have seen some of the

Plate 178. Bacillus Frumenti Phenolgenus

Photomicrograph of a corn bacillus which produces much gas. It causes sourness, decomposing the sugar into acids and carbonic acid gas. It is a very thin, short bacillus, having numerous flagella, which give it active motility. Isolated from imperfectly sterilized corn. It is an anaerobe. Magnified 1,200 diameters.

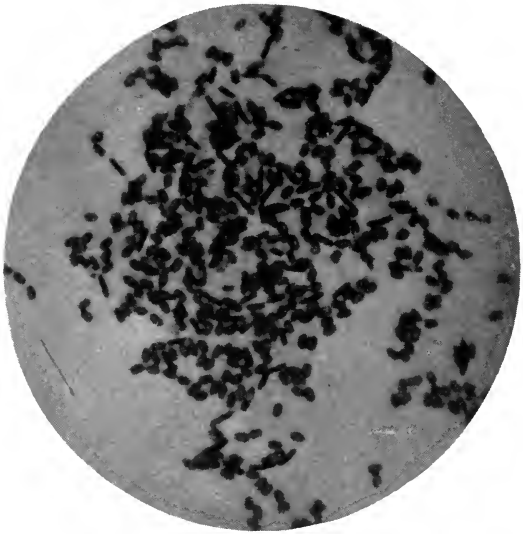


Plate 179. Bacillus Frumenti Phenolgenus

Photomicrograph of the spore-forms of the gas forming corn bacillus shown in plate 178. The spores are small and centrally located in the cells, and are heat resisting, although not as much, so as those shown in plate 177. Stained lightly with carbol fuchsine. Magnified 1,200 diameters.

reports from the agricultural chemists, in which they claim to have found salicylic acid in canned corn, and we mention the results we obtained with this bacillus as a possible source of phenol-like bodies sometimes found in canned corn.

WHAT CAUSED CANS OF CORN TO BURST.

NATIONAL CANNERS' LABORATORY,
Aspinwall, Pa.

GENTLEMEN:—We are sending you by express today a number of cans of corn, some of the cans being swollen and some bursted at the seam. We desire that you analyze the contents of these cans and determine the cause of their swelling and bursting. They were supposed to have received 250° Fahr., for the regular length of time.

The point which we are particularly anxious to learn is whether the swelling and bursting of these cans is due to imperfect sterilization or to a leaky condition of the cans. We have gathered from your bacteriological work along these lines that it is possible to determine from the nature of the bacteria present in canned goods whether they are there because of incomplete sterilization or whether they had gained entrance through a leak in the can.

Kindly investigate this matter thoroughly and let us have your report at the earliest possible moment. Thanking you in advance and awaiting your reply, we are,

Yours very truly,

_____.

The two cases of swelled and bursted cans of corn were received at the National Canners' Laboratory and report on same is here submitted:

A large per cent of the cans were burst and the contents gone. Some of the cans were burst on the side seam, some at the tops and bottoms and others had the tops and bottoms completely torn off, not where they were soldered, but where they were bent. The pressure necessary to produce this condition of affairs must have been enormous, probably 35 or 40 pounds to the square inch. There were also quite a number of cans which were swelled at both ends and from general appearance did not leak. A test was made with pressure on one of these swelled cans as follows: A hole was cut in the cap and the putrefied corn was shaken out and a piece of pipe was attached to the can and soldered perfectly tight and this pipe was connected with a steam autoclav and the pressure was raised to 30 pounds without bursting the can. There was no evidence of

any leak in the can. A microscopical examination of the seams showed no imperfections.

In order to determine whether these cans had spoiled from bacteria which had not been destroyed in the sterilizing process, or by bacteria which had gained entrance through some possible leak, it was necessary to isolate and study the nature of the germs present, and then form definite conclusions from the results of the bacteriological work. A number of Petri dishes, about thirty in all, were streaked with the juice of the corn from a half dozen swelled cans and these were placed in the incubator. A large number of tubes containing 2 per cent glucose agar and 2 per cent glucose bouillon were also inoculated at the same time, and these were placed

Plate 180

Photograph of a can which had burst from the pressure of gas generated in the corn by anaerobic spore-bearing bacteria. One of these cans was tested up to 30 pounds pressure, so that the power necessary to burst the seam and split the top and bottom must have been enormous.

in an anaerobic culture apparatus. The juice from the corn was obtained from the swelled cans under aseptic conditions in the following manner: A Bunsen flame was held so that it would strike the tin and a hole was punched through the tin with a sterilized awl directly in the flame. In every case the evolution of gas was enormous and took fire in some cases, which showed the presence of hydrogen. Test papers also showed the presence of phosphoretted and sulphuretted hydrogen; the odor was abominable. With a platinum needle previously sterilized to whiteness in the flame, transfers were made of the corn juice to the tubes of agar and bouillon previously mentioned. It often happens that the bacteria which produce such large quantities of gas belong to the anaerobic species; that is to say, they will not grow in the presence of atmosphere, the oxygen in the atmosphere being poisonous to them; therefore it is

necessary to entirely exclude oxygen, either by replacing it with another gas, such as hydrogen, or by absorbing it with chemicals, such as pyrogallic acid neutralized with sodium hydroxide.

Anaerobic bacteria, as a rule, are found in the soil and upon vegetable and decomposing organic matter. They are generally spore-bearing organisms and are *not freely* distributed in the air and are not likely to gain entrance to a can through a leak. Whenever canned vegetables are spoiled by bacteria which gain entrance through leaks in the cans there are two or three varieties, one or more of which are always present. In fact, the lactic acid bacteria have been found in all leaks so far examined in the laboratory.

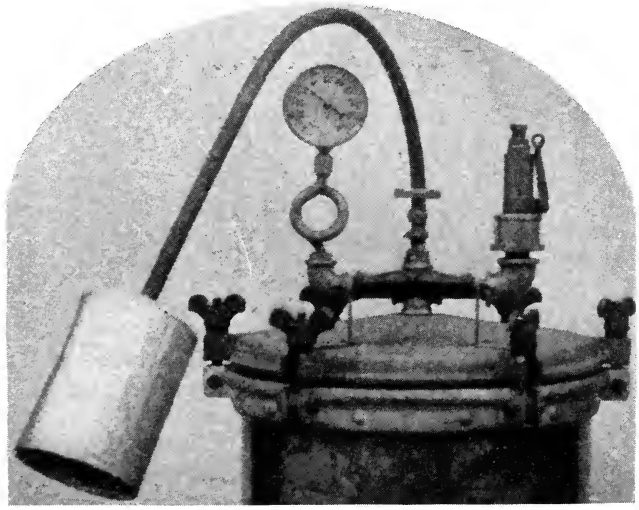


Plate 181

Photograph showing method of testing the can. The can shown was a "swell" containing corn. The can was opened, the spoiled corn was washed out and a pipe was soldered in the opening and connected with a steam autoclay and the pressure run up to 30 pounds without bursting the can or showing any leak, thus proving that the soldering was good and that the putrefaction of the corn was due to bacteria which had not been destroyed in the process of sterilization.

An examination was made of the juice from the cans in question, and no lactic acid or acetic acid bacteria, no molds, yeasts or micrococci could be detected with the microscope, but in all cases there were present large numbers of motile bacteria. Each can seemed to have a pure culture of these germs, although there were two distinct species, in the different cans, one more actively motile than the other. The Petri dishes which were inoculated with the juice were examined from time to time, but in no case was a single colony of bacteria obtained. These Petri dishes were incubated at 98 degrees Fahrenheit in the presence of free atmosphere. The results were far different with the tubes of glucose agar and bouil-

lon in the anaerobic apparatus. The agar was literally split all to pieces by the force of the gas generated and the glucose bouillon fermented freely. The cultures of the bacteria in all cases were pure, although there were two different species.

DESCRIPTION OF BACTERIA.

Plate shows young, vegetating rods which are stained by a special method to demonstrate the organs of locomotion. The rods are thin, three to five microns long and 0.3 to 0.5 of a micron wide, curly resembling tetanus. (A micron is equal to .025 of an

Plate 182

Photomicrograph, showing the curly flagella of the anaerobic Bacillus Butyricus Frumenti, obtained from a can of swelled corn. Some of the rods have the terminal spores and still retain their full equipment of flagella. This view was taken from a slide preparation specially stained, obtained from a twenty-four hours' growth on 2 per cent. glucose agar. Photographed through the microscope, using a 2 mm. oil immersion objective and acetylene radiant. Magnified 1,200 diameters.

inch.) In addition to the ordinary flagella there are scattered out the coverglass preparation great twisted bodies called by some authors "Giant Whips." These whips are different from similar bodies (described by various authors), in that they seem to have a small, round cell at the end. This cell is about 0.8 of a micron in diameter. It is not uncommon to find places where a large number of these cells are arranged in a mass with the "giant whips" extending outward, resembling spirochaetes.

Plate No. 5 shows the free spores and also spores formed at the end of the bacilli, which gives them the appearance of drumsticks or screw eyes. The spores are larger in diameter than the

rod forms. They are round, measuring from 1 to 1½ microns in diameter. These spores are very resistant to high temperatures on account of their thick walled membranes.

Plate No. 185 shows a growth of this organism in 2 per cent glucose agar. The agar is split in numerous places by the gas formed. This germ produces butyric acid, mercaptan and indol, and we have given it the name of "Bacillus Butyricus Frumenti," a name which indicates its origin in corn.

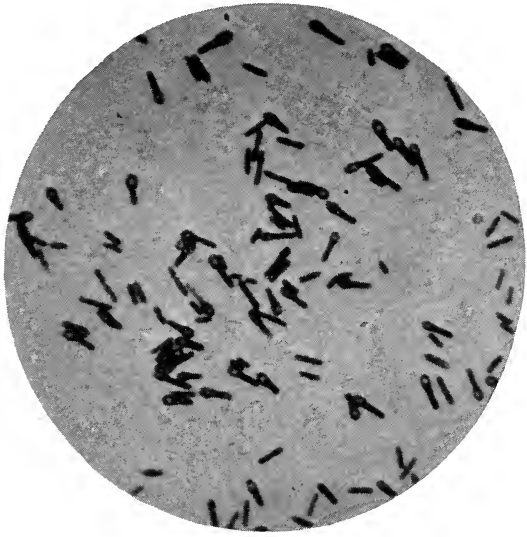


Plate 183

Photomicrograph of the spores of Bacillus Butyricus Frumenti, an anaerobic bacillus obtained from a swelled can of corn. This germ produces a terminal round spore which gives the rod the appearance of a drumstick or screweye and greatly resembles Tetanus. The spores are thick-walled and are very resistant to high temperatures. From culture on 2 per cent. glucose agar. Stained lightly with carbol fuchsine. Magnified 1,200 diameters.

Plate No. 186 shows a young vegetating form of another bacillus similar in some respects to the species just described, being both anaerobic and spore-bearing. From its nature it greatly resembles Bacillus Butyricus (Prazmowski). It is a beautifully flagellated bacillus, with rods of varying length and 1 micron wide and called "Amylobacter," from the fact that when grown upon media containing starch the cells will stain blue with iodin. It is more actively motile than the other species described and the spores are located generally in the center of the rods, as shown in plate No. 8.

This organism has the power to cause the fermentation of cellulose, but we believe that it is different from Bacillus Amylobacter used in pure cultures in the ripening of cheese.

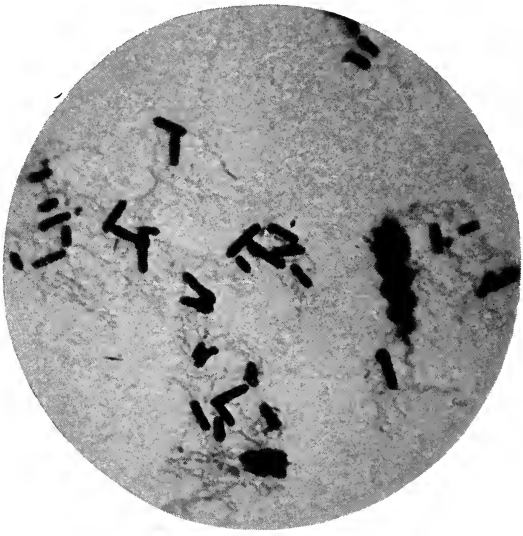


Plate 184

Photomicrograph of Bacillus Butyricus Frumenti, showing ordinary flagella and also a bunch of giant whips greatly resembling a bunch of hair. This is an obligative anaerobic bacillus found in corn and was obtained from a swelled can of corn. The pressure of gas created by this organism is enormous, sufficient to burst the cans. Stained by our special method from a young growth on 2 per cent. glucose agar. Photographed through a 2 mm. oil immersion objective using acetylene radiant. Magnified 1,200 diameters.

Plate 185

Photograph of a test tube culture of the anaerobic Bacillus Butyricus Frumenti, on 2 per cent. glucose agar. The force of gas was sufficient to split the agar in many places, forming pockets filled with gases of various kinds, such as sulphuretted hydrogen, phosphoretted hydrogen, hydrogen, etc. The growth of this germ in media containing no sugar is not as free in gas formation.

Besides butyric acid it excretes some foul substances, such as indol and sulphuretted hydrogen. The odor of canned corn indicating decomposition by this agency is abominable. It took several hours to remove the malodorous gases from the laboratory.

These two species were the only ones found in the swelled cans, which gave no indication of leaking. These species are not contaminations from the atmosphere. They were not destroyed in the

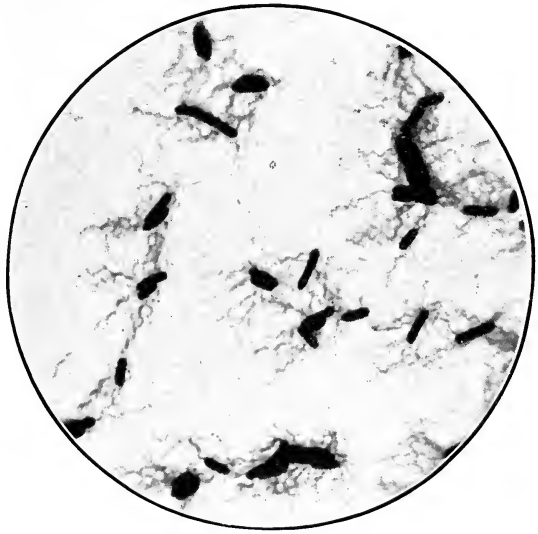


Plate 186

Photomicrograph of Bacillus Butyricus Amylobacter, an anaerobic bacillus which when grown on substances containing starch will stain blue with iodine. The flagella are very curly and were demonstrated by our own special method, from a 24 hours' growth on 2 per cent. glucose agar which had been inoculated from the juice of corn in a swelled can. This organism is frequently found in decomposing vegetables and organic matter, and is not found in the air. Its habitat is probably the soil. Magnified 1,200 diameters.

sterilizing process; they did not gain entrance to the corn through any leaks; they would not be growing alone in pure cultures in case they had by chance gained entrance to the can. They are spore-bearing organisms and there were *no non-spore-bearing organisms present.* As stated previously, lactic acid and acetic acid, molds, yeasts and micrococci are the species which are freely distributed in the atmosphere, and some of these would most certainly have been present in case the cans had leaked. There can be no other conclusion than that the sterilizing process was insufficient to prevent the growth and multiplication of anaerobic bacteria present in the corn itself. It might be added that these two species de-

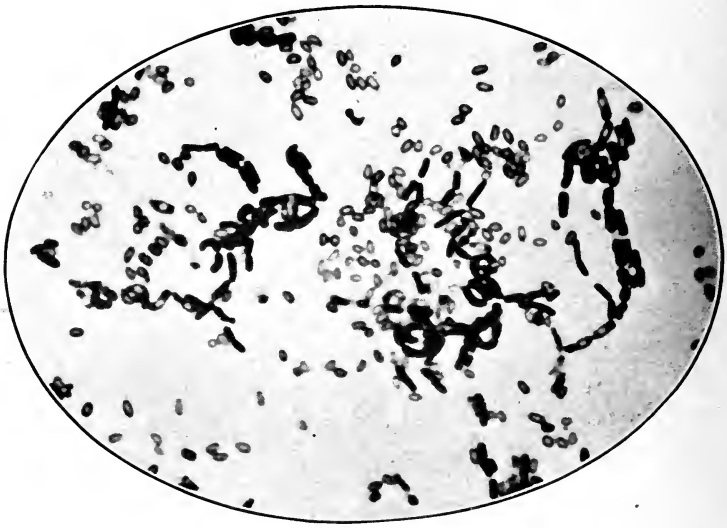


Plate 187

This beautiful photomicrograph shows the free spores and the rods containing spores of Bacillus Butyricus Amylobacter. The spores are generally formed in the center of the rods which cause them to swell in the middle like spindles, hence they belong to the type called "clostridium." The spores are not easily destroyed by heat and may live in corn which has received a temperature of 250 degrees for one hour. Stained with carbol fuchsine. Magnified 1,500 diameters.

scribed are commonly found associated with corn. A similar organism to the one shown in plate 183 is often found in spoiled canned peas, but the rods are much more slender and the spore is ellipsoidal instead of round.

FINIS.

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