LABORATORY COURSE
IN
BACTERIOLOGY

GORHAM

.. LIBRARY ..

**Connecticut
Agricultural College.**

VOL. 9778
CLASS NO. 616.01 967.
COST 1 L3
DATE Dec. 23 1903

616.01
G67
9778

58
63
1901

A LABORATORY COURSE
IN
BACTERIOLOGY

For the Use of Medical, Agricultural, and
Industrial Students

BY
FREDERIC P. GORHAM, A.M.

Associate Professor of Biology, Brown University; Bacteriologist,
Health Department, Providence, R. I.

WITH 97 ILLUSTRATIONS

PHILADELPHIA AND LONDON
W. B. SAUNDERS & COMPANY

1901

616.01
G67

COPYRIGHT, 1901,
By W. B. SAUNDERS & COMPANY.

9778.

ELECTROTYPED BY
WESTCOTT & THOMSON, PHILADA.

PRESS OF
W. B. SAUNDERS & COMPANY.

PREFACE.

BACTERIOLOGY is essentially a laboratory study. It is only by actual laboratory work that it can be taught in such a manner as to serve any useful purpose. It is also a subject of very general scientific interest. Courses in bacteriology are no longer confined to the medical schools, but are being introduced into colleges and agricultural and industrial schools. This volume has been prepared as a guide to the practical details of laboratory work. It is intended to present the subject in such a general way as to lay a broad foundation for later specialization in any branch of bacteriology. By a judicious selection the course can be made to conform to the requirements of medical, agricultural, or industrial students.

BROWN UNIVERSITY, August, 1901.

CONTENTS.

CHAPTER I.

	PAGE
MICROSCOPIC EXAMINATION OF BACTERIA	11
Manipulation of the Microscope, 11.—Measurement of Bacteria, 11.—Examination of Living Bacteria, 13.—Ordinary Examination, 13.—Hanging Drop, 15.—Examination of Stained Bacteria, 16.—Ordinary Stains, 16.—Gram's Stain, 19.—Staining Bacteria in Tissues, 20.	

CHAPTER II.

MORPHOLOGY OF BACTERIA	22
Demonstration of Form, 22.—Demonstration of Motion, 24.—Staining Flagella, 25.—Demonstration of Capsules, 31.	

CHAPTER III.

REPRODUCTION OF BACTERIA	34
Division, 34.—Spores, 35.—Staining Spores, 36.—Germination of Spores, 38.	

CHAPTER IV.

CLASSIFICATION OF BACTERIA	39
--------------------------------------	----

CHAPTER V.

STERILIZATION	43
Steam, 46.—Autoclave, 46.—Hot-air, 47.	

CHAPTER VI.

PREPARATION OF CULTURE-MEDIA	50
Bouillon, 50.—Gelatin, 50.—Agar, 50.—Media from Meat-extracts, 55.—Potato, 55.—Dextrose, Lactose, and Saccharose Bouillon, 56.—Milk and Litmus Milk, 57.—Blood serum, 59.	

CHAPTER VII.

	PAGE
CULTURES OF BACTERIA	60
Bouillon Cultures, 60.—Gelatin or Agar Cultures, 61.—Potato and Blood-serum Cultures, 66.—Plate Cultures, 66.—Impression or Adhesive Preparations of Colonies, 75.—Cultures in the Fermentation-tube, 76.—Anaërobic Cultures, 78.—Demonstration of Liquefying Ferment, 80.—Isolation of Species, 81.	

CHAPTER VIII.

DETERMINATION OF SPECIES	83
Morphology and Life-history of a Species, 83.—Determination of the Name of a Species, 95.—Classification of Bacteria by Groups, 107.—Classification of Water Bacteria by Groups, 112.	

CHAPTER IX.

BACTERIAL ANALYSIS OF WATER, MILK, AIR, AND SOIL . . .	114
Water Analysis, 114.—Milk Analysis, 120.—Bacteria in the Air, 121.—Bacteria in the Soil, 122.	

CHAPTER X.

PATHOGENIC BACTERIA	124
Pyogenic Organisms, 125.—Gonococcus, 129.—Anthrax, 130.—Glanders, 133.—Diphtheria, 135.—Influenza, 141.—Typhoid and Colon Bacilli, 144.—Pneumonia, 150.—Tuberculosis, 151.—Actinomycosis, 158.—Malaria, 159.	

APPENDIX	163
Bacterial Measurements by Photography, 163.—Moulds and Yeasts, 165.—Stains and Reagents used in the Study of Bacteria, 170.—Table of Synonyms, 175.	

INDEX	183
-----------------	-----

A LABORATORY COURSE
IN
BACTERIOLOGY

A LABORATORY COURSE IN BACTERIOLOGY.

CHAPTER I.

MICROSCOPIC EXAMINATION OF BACTERIA.

I. MANIPULATION OF THE MICROSCOPE.

1. Examine mounted slides of bacteria with the low-power ($\frac{2}{3}$ inch) and high-power ($\frac{1}{6}$ inch) objectives and with different eyepieces.

2. Manipulate the condenser, diaphragm, and mirror, in order to ascertain what combination gives the best result.

3. (a) Affix the oil-immersion lens ($\frac{1}{12}$ inch) to the microscope.

(b) Place a drop of immersion oil on the cover-glass.

(c) Bring the lens to a focus in the drop of oil.

(d) Again manipulate condenser, diaphragm, and mirror to determine what combination now is best.

II. MEASUREMENT OF BACTERIA.

The most accurate method of measurement is by photography ;¹ but as this requires special appa-

¹ See Appendix, page 163.

ratus, the following fairly accurate methods are recommended :

1. First Method.

(a) Place a micrometer eyepiece in the microscope.

(b) Examine the bacteria to be measured, and record their lengths in divisions of the eyepiece micrometer.

(c) Remove the slide of bacteria.

(d) Place a stage micrometer on the stage of the microscope, and determine the relation of the divisions of the eyepiece micrometer to the divisions of the stage micrometer.

(e) The length of the divisions of the stage micrometer is a fixed quantity (usually $\frac{1}{100}$ mm.); therefore we have the equations :

Length of bacteria = x divisions of eyepiece micrometer ;

x divisions of eyepiece micrometer = y divisions of stage micrometer ;

1 division of stage micrometer = $\frac{1}{100}$ mm.

From these equations we can readily determine the length of the bacteria in millimeters. The unit of length for microscopic measurements is the thousandth part of a millimeter ; it is called a micron or micromillimeter, and is designated by the Greek letter μ . Therefore in the above equations we can substitute for $\frac{1}{100}$ mm. 10 μ , and write the result in so many μ .

2. Second Method.

(a) Adjust the "camera lucida" to the microscope.

(*b*) Draw by means of the camera the exact size of the bacteria to be measured.

(*c*) Remove the slide of bacteria, and replace it with the stage micrometer.

(*d*) Over the figure of the bacteria now draw the scale of the stage micrometer, and read off directly the size of the bacteria in divisions of the stage micrometer.

III. EXAMINATION OF LIVING BACTERIA.

I. Ordinary Examination.

(*a*) Place a drop of water on a clean slide.

(*b*) Pass the cotton plug of the tube containing the culture to be examined through the flame ; ex-

FIG. 1.—Method of holding tubes during inoculation (McFarland).

tinguish the ignited cotton by blowing or pinching out the flame.

(*c*) Hold the test-tube containing the culture be-

tween the thumb and finger of the left hand, allowing the lower end of the tube to rest on the back of the hand.

(*d*) Hold a straight platinum needle between the thumb and forefinger of the right hand, and sterilize it by heating red-hot. Allow it to cool.

FIG. 2.—Platinum needles for transferring bacteria; made from No. 27 platinum wire inserted in glass rods.

(*e*) Grasp the cotton plug between the third and fourth finger of the right hand, and remove it; insert the platinum needle, and transfer an exceedingly minute portion of the culture of bacteria to the drop of water.

(*f*) Return the cotton plug to the tube, and sterilize the needle.¹

(*g*) Place a clean² cover-glass over the drop of water, and examine with the $\frac{1}{6}$ inch objective.

¹ The needle should invariably be heated before and after using. If this practice is not carefully followed, cultures will be contaminated, and perhaps pathogenic organisms spread about the room. It is well also to pass the handle of the needle through the flame before beginning work each day.

² Cover-glasses and slides may be cleaned in the following solution :

Potassium bichromate,	6 gm. ;
Concentrated sulphuric acid,	6 c.c. ;
Water,	100 c.c.

Wash in water and store in alcohol.

Or boil the cover-glasses in sulphuric acid, wash in

(h) Manipulate condenser, diaphragm, and mirror to determine the best adjustment for the examination of unstained bacteria.

2. Examination in the "Hanging Drop."

FIG. 3.—A "concave slide" with "hanging drop" (McFarland).

FIG. 4.—A slide with cell for hanging drop. The ring may be made of glass or of zylonite, and is cemented on the slide with Canada balsam.

(a) Paint a ring of vaselin around the hollow in
 water, and keep in alcohol; or wash in strong nitric acid for some time, rinse in water, and store in alcohol. Very often simply passing them several times through a Bunsen flame will clean them sufficiently.

a "concave slide;" or use a slide on which is cemented a small glass ring, and vaselin the top of the ring.

(b) On the centre of a clean cover-glass place a small drop of water.

(c) With a sterile platinum needle add to the drop of water a very small portion of the culture to be examined.

(d) Invert the slide over the cover-glass, so that the drop of water is covered by the concavity or is inside the glass ring, but does not touch the sides of either; press down so that the chamber is sealed tight by the vaselin.

(e) Invert carefully and examine.

The hanging-drop examination is for the purpose of determining the motility of bacteria or for watching their reproduction. The preparation may be kept for examination from day to day without loss by evaporation.

IV. EXAMINATION OF STAINED BACTERIA.

I. Ordinary Stains.

(a) Prepare a clean cover-glass.¹

(b) Place a drop of water on the glass.²

(c) With a sterile platinum needle transfer a minute portion of a culture to the drop of water

¹ See footnote, page 14, for directions for cleaning covers.

² If the cultures are in bouillon or other fluid, it is often unnecessary to use the drop of water in spreading them on the cover-glass.

and spread uniformly over the surface of the cover-glass.¹

(d) Allow the film to dry.

(e) When dry pass the cover-glass, smeared sur-

FIG. 5.—Cornet's cover-glass staining forceps.

FIG. 6.—Stewart's cover-glass forceps.

FIG. 7.—Kalteyer's cover-glass staining forceps.

face upward, three times through a Bunsen or alcohol flame at about the rate of the pendulum of a clock.

The heat coagulates the albuminous material

¹ The cover may be held, while staining, in one of the special forceps devised for the purpose.

around the bacteria and fixes them firmly to the glass.

(*f*) Place a drop of stain ¹ on the cover-glass large enough to cover the film. Allow it to stain for from two to ten minutes; the length of time depends on the stain, the strength of the staining solution, and the kind of bacteria.²

FIG. 8.—Bottles for stains.

(*g*) Wash in water, dry the unsmeared side on filter-paper, mount, film side down, in a drop of water on a clean slide, and examine with the $\frac{1}{6}$ inch objective.

¹ Almost any of the anilin stains may be employed. Gentian-violet, basic fuchsin, and methylene-blue are those most commonly used. For methods of preparing these staining solutions, see Appendix, page 170.

² It very rarely happens that bacteria are over-stained. But if such is the case, either a new film must be prepared or the stain drawn with weak acetic acid (1 : 1000), or the cover-glass swept through 1 per cent. sulphuric acid. If the film is not sufficiently stained, repeat the staining process.

(h) If the bacteria are evenly distributed¹ and properly stained, dry both sides thoroughly between filter-paper and mount, film side down, in a drop of Canada balsam. Label and preserve.

2. Gram's Stain.

The value of this method depends on the fact that the mycoprotein of certain bacteria forms with an anilin dye and an iodid a compound insoluble in alcohol. There are many bacteria in which such an insoluble compound is not formed, and this method consequently has considerable diagnostic value.

(a) To a drop of water on a clean cover-glass add a very small amount of a culture of *Bacillus subtilis*.

(b) To the same drop add a very small amount of a culture of *Bacillus vulgaris*, or any culture that does not stain by this method. ~

(c) Dry, fix, and stain for five minutes in anilin gentian-violet.²

(d) Wash in water.

(e) Treat with Gram's solution³ for one minute.

¹ In a successful preparation the bacteria are evenly and not too thickly distributed over the surface. If too many bacteria are present, either a smaller amount of the culture must be used, or a little from the first cover-glass must be added to another drop of water on a second cover-glass, and so on until the proper dilution is reached.

² See Appendix, page 171.

³ Gram's solution :

Iodin,	1 part ;
Potassium iodid,	2 parts ;
Distilled water,	300 parts.

(*f*) Wash in 95 per cent. alcohol until no more color comes away.

(*g*) Dry and contrast-stain in safranin¹ (thirty seconds), or Bismarck-brown² (two to three minutes), or eosin³ (one minute).

(*h*) Wash, dry, and mount.

3. Staining Bacteria in Tissues.

(1) First Method.

(*a*) Harden the tissue in absolute alcohol or Zenker's fluid.⁴

(*b*) Dehydrate, embed, and section by the usual methods.

(*c*) Stain as directed for cover-glass preparations, but a little more deeply.

(*d*) Draw the color with dilute acetic acid (1 : 1000) until the bacteria alone are stained.

(*e*) Contrast-stain in eosin or any stain not requiring acid for differentiation.

(*f*) Clear and mount.

(2) Second Method.

Stain the sections by Gram's method as follows :

(*a*) Stain in anilin gentian-violet for five minutes.

(*b*) Wash in water.

(*c*) Treat with Gram's solution for one minute.

(*d*) Wash in 95 per cent. alcohol until no more color comes away.

¹ Stock solution is a 1 per cent. solution of safranin in equal parts of methylated spirit and water. For use, dilute with 5 parts of water.

² Saturated solution in equal parts of alcohol and water.

³ Aqueous solution, 1 : 1000.

⁴ See Appendix, page 173.

(e) Wash in water.

(f) Contrast-stain in an aqueous solution of eosin (1 : 1000) for one-half to one minute.

(g) Wash in 60 per cent. alcohol for thirty seconds.

(h) Wash in absolute alcohol for thirty seconds.

(i) Clear in xylol and mount in balsam.

(3) **Third Method.**

(a) Stain in Kuhne's methylene-blue¹ for one-half to one hour, or in carbol-thionin-blue² for five minutes.

(b) Wash in water.

(c) Treat with 0.5 per cent. acetic acid till pale green.

(d) Wash in water, 60 per cent. alcohol, and absolute alcohol, each for thirty seconds.

(e) Contrast-stain as in above methods, clear, and mount.

(4) **Fourth Method.**

(a) Stain the dried preparations in a dilute aqueous solution of methylene-blue.

(b) Wash in water and dry.

(c) Stain in aqueous eosin solution (1 : 1000) for one to one and a half minutes.

(d) Dehydrate, clear, and mount.

¹ See Appendix, page 171.

² See Appendix, page 171.

CHAPTER II.

MORPHOLOGY OF BACTERIA.

BACTERIA are minute, unicellular, vegetable organisms. They consist of a sharply defined mass of protoplasm which reacts to anilin stains very much like the nuclei of other cells, and outside of this a more or less well-developed envelope. They are classified according to their form into three main groups, the spherical cocci, the rod-shaped bacilli, and the curved or spiral spirilla.

FIG. 9.—Diagram illustrating the morphology of the cocci : *a*, coccus or micrococcus ; *b*, diplococcus ; *c*, *d*, streptococci ; *e*, *f*, tetragenococci or merismopedia ; *g*, *h*, modes of division of cocci ; *i*, sarcina ; *j*, coccus with flagella ; *k*, staphylococci (McFarland).

I. Demonstration of Form.

(*a*) Make hanging-drop and stained preparations from cultures of cocci, bacilli, and spirilla.

(*b*) Examine with the $\frac{1}{6}$ inch or with the oil-immersion lens.

In the hanging-drop preparations are the individual bacteria spherical, rod-shaped, or spiral? If rod-shaped, are the ends pointed, rounded, or square? Are the bacteria motile?¹ Are they single

FIG. 10.—Diagram illustrating the morphology of the bacilli: *a*, *b*, *c*, various forms of bacilli; *d*, *e*, bacilli with flagella; *f*, chain of bacilli, individuals distinct; *g*, chain of bacilli, individuals not separated (McFarland).

FIG. 11.—Diagram illustrating the morphology of the spirilla: *a*, *b*, *c*, spirilla; *d*, *e*, spirochæta.

or united in pairs, fours, irregular masses, or chains? Preserve the hanging-drop preparations for further study.

(*c*) Repeat these observations on the stained preparations.

¹ See § II., page 24.

Are the individuals stained uniformly or irregularly, deeply or faintly? In the rods are the ends more deeply stained than the centers (polar staining)?

(*d*) Measure the various kinds and make drawings of them.

II. Demonstration of Motion.

(*a*) To a small drop of water on a cover-glass add a very little carmine or Bismarck-brown.

FIG. 12.—*Bacillus suipestifer*, showing flagelia.

(*b*) Mount in the same manner as a hanging-drop preparation.

(*c*) Examine with the $\frac{1}{6}$ inch objective.

Are the bits of pigment in motion? Do they change their position relative to one another, or do they dance about in one place? This is the so-called Brownian movement.

(*d*) Examine the hanging-drop preparations of the last section in reference to this movement.

Do they all show the Brownian movement? Are some actively swimming about, changing their position in relation to one another? All bacteria exhibit the Brownian movement, but certain ones are motile of themselves. They possess organs of locomotion or flagella, lash-like appendages, by the movements of which they propel themselves along. The flagella may be very numerous, extending from all sides of the cell, or they may be collected in a tuft at one end, or there may be a single one or a pair (peritrichous, lophotrichous, monotrichous, amphitrichous). The flagella may be demonstrated by appropriate methods of staining.

III. Staining Flagella.

I. Pitfield's Method.

(*a*) Prepare a mordant as follows:

Tannic acid (10 per cent. solution, filtered),	10 c.c. ;
Corrosive sublimate (saturated aqueous solution),	5 c.c. ;
Alum (saturated aqueous solution),	5 c.c. ;
Carbol-fuchsin ¹	5 c.c.

Allow to stand and draw off the clear fluid. The mordant will keep one or two weeks.

¹ See Appendix, page 172.

(b) Prepare a stain as follows :

Alum (saturated aqueous solution),	10 c.c.;
Gentian-violet (saturated alcoholic solution),	2 c.c.

The stain will keep two or three days.

(c) On an *absolutely* clean cover-glass¹ place a drop of distilled water.

(d) With a sterile platinum needle add to the drop of water, without stirring, a very small portion of an actively motile culture.

(e) Stand on a water-bath at 60° C. for one-half hour.

(f) Treat the film with the cold mordant for twenty-four hours, or with hot mordant (steaming, but not boiling) for three minutes.

(g) Wash thoroughly in running water and dry.

(h) Treat with the stain the same as directed for the mordant.

(i) Wash, dry, mount, and examine with the oil-immersion lens.

2. Modification of Pitfield's Method.

(a) Prepare solution A as follows :

Alum (saturated aqueous solution),	10 c.c.;
Gentian-violet (saturated alcoholic solution),	1 c.c.

¹ See footnote, page 14.

(b) Prepare solution B as follows :

Tannic acid,	1 gm. ;
Distilled water,	10 c.c.

(c) Filter when cold, mix the two solutions, and use immediately.

(d) Prepare films as before.

(e) Treat with the above mixture, gently heating until it almost boils, then set aside for a minute.

(f) Wash, dry, and mount.

FIG. 13.—*Microspira comma*, showing the flagella ; $\times 1000$ (Günther).

3. Löffler's Method.

(a) Prepare a mordant as follows :

Tannic acid (20 per cent. solution), filtered,	10 c.c. ;
Ferrous sulphate (cold, saturated solution), filtered,	5 c.c. ;
Fuchsin (saturated alcoholic solution),	1 c.c.

Filter each time before using. This mordant will keep two or three days.

(*b*) Prepare films as before.

(*c*) Dry and pass once through the flame.

(*d*) Cover the film with the mordant, and warm over the flame for a few seconds only.

FIG. 14.—*Bacillus typhosus*, from an agar culture six hours old, showing the flagella stained by Löffler's method; $\times 1000$ (Fränkel and Pfeiffer).

(*e*) Wash in water, dip in absolute alcohol, and again wash in water.

(*f*) Cover the film with anilin-water fuchsin,¹ and warm over the flame from three to four minutes, taking care that the solution steams, but does not boil.

¹ See Appendix, page 171.

(*g*) Wash in water, dry, mount, and examine with oil-immersion lens.

4. Modification of Löffler's Method.

(*a*) Prepare a mordant as follows :

Tannic acid (20 per cent. solution),	10 c.c. ;
Ferrous sulphate (saturated solution),	10 c.c. ;
Logwood solution (1 gram boiled in 8 c.c. of water and filtered),	3-4 c.c.

(*b*) Prepare a stain as follows :

Anilin-water, ¹	100 c.c. ;
Sodium hydrate (1 per cent.),	1 c.c. ;
Methylene-violet or methylene-blue or fuchsin,	4-5 gm.
Filter.	

(*c*) Prepare films as before.

(*d*) Heat with the mordant until steam rises, and then move over flame for one minute.

(*e*) Wash in water, and if mordant does not disappear, in absolute alcohol.

(*f*) Dry and heat with the stain until steam rises, then leave in the warm stain one minute.

(*g*) Wash, dry, and mount.

¹ See Appendix, page 171.

5. Van Ermengem's Method.¹

(a) Prepare solution A as follows :

Osmic acid (2 per cent. solution), 1 part ;

Tannic acid (10 to 25 per cent. solution), 2 parts.

To each 100 c.c. of the tannic acid add 4 or 5 drops of glacial acetic acid.

FIG. 15.—Bacterium Fäschingii in blood ; $\times 1000$ (Fränkel and Pfeiffer).

¹ Various modifications of Van Ermengem's method have been suggested. See *Centralbl. f. Bakt.*, Erste Abteilung, Bd. xxvii., Nos. 16–17, p. 597 ; and *Lancet*, vol. ii., No. 14, p. 874, 1898.

(b) Prepare solution B as follows :

Gallic acid,	5 gm. ;
Tannic acid,	3 “
Fused acetate of sodium or potassium,	10 “
Distilled water,	350 c.c.

(c) Prepare films as before.

(d) Place in solution A for one-half hour ; or, if the solution is warmed to 60° C., five minutes are sufficient.

(e) Wash thoroughly in water, then in alcohol, then again in water.

(f) Place for two minutes in solution of nitrate of silver (0.25–0.5 per cent.).

(g) Without washing, place in solution B for one and one-half to two minutes, using fresh solution for each preparation.

(h) Wash thoroughly in water, and examine in water. If the flagella are not sufficiently stained, begin again at (f).

Always change the silver nitrate solution as soon as any precipitate appears.

IV. Demonstration of Capsules.

In certain forms the envelopes surrounding the cells can be stained as follows :

I. Welch's Method.¹

(a) Prepare films without using water.

(b) Place in glacial acetic acid for a few seconds.

(c) Remove the acid with filter-paper.

¹ *Bulletin of the Johns Hopkins Hospital*, p. 128, December, 1892.

(*d*) Wash in anilin-water gentian-violet repeatedly until all the acid is removed.

(*e*) Wash in a $\frac{1}{2}$ per cent. solution of sodium chlorid and examine in the same solution.

2. Johne's Method.

(*a*) Prepare films as usual.

(*b*) Warm in a 2 per cent. solution of gentian-violet until steam arises.

FIG. 16.—*Bacterium pneumoniae* of Friedländer, from the expectoration of a pneumonia patient; $\times 1000$ (Fränkel and Pfeiffer).

(*c*) Wash in water.

(*d*) Place in 2 per cent. acetic acid for six to ten seconds.

(*e*) Wash in water.

(*f*) Dry and mount in balsam.

3. Boni's Method.¹

(a) Prepare a solution as follows :

White of one egg ;

Glycerin, 50 c.c. ;

Formalin, 2 drops.

Shake together and filter.

(b) Prepare films of the organism to be studied, using the above solution in place of water.

(c) Spread very thin, and heat over flame until the glycerin is evaporated.

(d) Stain in Ziehl's carbol-fuchsin² for twenty to thirty seconds.

(e) Wash in water and dry with filter-paper.

(f) Contrast-stain in Löffler's methylene-blue³ for four to six minutes.

(g) Wash, dry, and mount in balsam.

¹ *Centralbl. für Bakteriologie*, Erste Ab., Band xxviii., No. 20, p. 705.

² See Appendix, page 172.

³ See Appendix, page 171.

CHAPTER III.

REPRODUCTION OF BACTERIA.

THE common method of reproduction among the bacteria is by binary division. In some forms this is the only mode of reproduction known. Under favorable conditions an individual cell grows in length, a transverse constriction appears in the middle and gradually becomes deeper until two new cells are formed. Separation may be complete or there may be the formation of chains. In the spherical forms division may take place in one, two, or three planes, forming chains (streptococci), or groups of two (diplococci), irregular groups (staphylococci or micrococci), or cubical packets (sarcina). In the rod-shaped forms division takes place in but one plane, forming chains, pairs, or single individuals (Figs. 9 and 10).

Some bacteria, besides having the power of reproduction by division, form endogenous spores. These spores are developed from the plasma of the cell, and have a dense wall that protects them from injury by drying, enables them to withstand high temperatures, and also causes them to resist the action of stains.

I. Reproduction by Division.

(a) On a clean cover-glass place a large drop of bouillon.¹

¹ See page 50.

(b) Add to the bouillon a very small portion of a culture of *Bacillus subtilis*, or any chain-forming and spore-producing species.

(c) Mount as a hanging-drop preparation and place in the incubator.

(d) Examine at intervals of one-half hour.

Notice the elongation of the bacilli and the formation of chains.

(e) Inoculate a bouillon-tube as directed on page 60.

(f) Place in the incubator and make stained preparations at half-hour intervals.

Preserve both hanging-drop preparation and bouillon-tube for further examination.

II. Reproduction by Spores.

1. After the formation of chains has taken place in the above cultures, continue the examination at intervals. The formation of spores within the rods will be observed; finally, the rods will disappear and nothing but spores remain.

FIG. 17.—Diagram illustrating sporulation: *a*, bacillus enclosing a small oval spore; *b*, drumstick bacillus, with the spore at the end; *c*, clostridium; *d*, free spores; *e*, and *f*, bacilli escaping from spores.

2. Make stained preparations from the bouillon culture when rods and spores within the rods and spores alone are all present. Stain as follows:

III. Staining Spores.

(I) First Method.

(a) Prepare films in the usual manner.

(b) Stain in warm Ziehl's carbol-fuchsin¹ for twenty to thirty minutes, or in the same solution steaming, but not boiling, for five minutes.

(c) Wash in water.

(d) Dip in acid alcohol (70 per cent. alcohol, 97

FIG. 18.—*Bacterium anthracis*, stained to show spores ;
× 1000 (Fränkel and Pfeiffer).

c.c. ; hydrochloric acid, 3 c.c.) or in 1 per cent. sulphuric acid a few seconds and wash in water.

(e) Examine in water.

Spores should be red and rods unstained or faintly pink. If the spores are not sufficiently

¹ See Appendix, page 172.

stained, place again in the fuchsin. If the rods are still stained, wash longer in the acid alcohol.

(*f*) Dry and pass three times through the flame.

(*g*) Stain in Löffler's methylene-blue¹ for three to four minutes, or in gentian-violet² one minute, or in saturated aqueous solution of methylene-blue for half a minute.

(*h*) Wash, dry, and mount.

Spores should be red, rods blue.

(2) **Second Method.**

(*a*) Prepare films in the usual manner.

(*b*) Place in chloroform for two minutes.

(*c*) Treat with 5 per cent. chromic acid for one minute. The acid acts on the membranes of the spores and permits the entrance of the stain.

(*d*) Wash thoroughly in water.

(*e*) Stain in hot Ziehl's carbol-fuchsin for three minutes.

(*f*) Without washing decolorize in 5 per cent. sulphuric acid.

(*g*) Wash in water and stain in Löffler's methylene-blue¹ for two minutes or longer.

(*h*) Wash, dry, and mount.

(3) **Third Method.**

(*a*) Suspend the spore-bearing bacteria in a normal salt solution³ in a test-tube.

(*b*) Add an equal volume of Ziehl's carbol-fuchsin.

(*c*) Place the test-tube in boiling water for fifteen minutes.

¹ See Appendix, page 171.

² See Appendix, page 171.

³ See Appendix, page 173.

(*d*) Spread a loopful on a cover-glass; dry; fix.

(*e*) Decolorize in a $1\frac{1}{2}$ per cent. (by volume) solution of hydrochloric acid in alcohol.

FIG. 19.—*Bacillus chauvæi*, showing spores; $\times 1000$
(Fränkel and Pfeiffer).

(*f*) Wash in water and contrast-stain in Löffler's methylene-blue.

(*g*) Wash, dry, and mount.

(4) **To Observe the Germination of Spores.**

(*a*) Make a hanging-drop preparation, using sterile bouillon and some of the spores from the above culture.

(*b*) Place on a warm stage and watch the growth of rods from the spores.

(*c*) Inoculate a sterile bouillon-tube with a large number of spores from the above culture.

(*d*) Make stained cover-glass preparations from this at intervals of one-half hour.

CHAPTER IV.

CLASSIFICATION OF BACTERIA.

THE position occupied by the bacteria in the vegetable world is shown by the table on page 40.

The Bacteria or Schizomycetes are classified into families according to their form, and again into genera according to certain other characters. The following classification is that proposed by Migula in his *System der Bakterien*, 1900 :

BACTERIA, SCHIZOMYCETES.

EUBACTERIA, cells contain no sulphur granules or bacterio-purpurin.

1. Family COCCACEÆ, spherical forms.

Genus :

1. *Streptococcus*, non-motile ; cells divide in one plane.
2. *Micrococcus*, non-motile ; cells divide in two planes.
3. *Sarcina*, non-motile ; cells divide in three planes.
4. *Planococcus*, motile ; cells divide in two planes.
5. *Planosarcina*, motile ; cells divide in three planes.

2. Family BACTERIACEÆ, straight, rod-shaped forms without envelope.

Genus :

1. *Bacterium*, non-motile.
2. *Bacillus*, motile ; flagella over whole surface.
3. *Pseudomonas*, motile ; flagella polar.

3. Family SPIRILLACEÆ, curved, rod-shaped forms without envelope.

Genus :

1. *Spirosoma*, non-motile ; cells rigid.
2. *Microspira*, motile ; one, rarely two or three, polar flagella.
3. *Spirillum*, motile ; polar tufts of flagella.
4. *Spirochæta*, cells flexile.

4. Family CHLAMYDOBACTERIACEÆ, cells with envelopes.

Genus :

1. *Chlamydothrix*, unbranched threads ; cell-division in one plane.
2. *Crenothrix*, unbranched threads ; cell-division in three planes ; sheath visible.
3. *Phragmidiothrix*, unbranched threads ; cell-division in three planes ; sheath scarcely visible.
4. *Sphærotilus*, branched threads.

THIOBACTERIA, cells contain sulphur granules or bacterio-purpurin ; red or violet color, never green.

1. Family BEGGIATOACEÆ, thread-forming, without bacterio-purpurin.

Genus :

1. *Thiotrix*, attached threads ; non-motile.
2. *Beggiatoa*, unattached threads ; motile.

2. Family RHODOBACTERIACEÆ, cells contain bacterio-purpurin and sulphur granules ; red or violet.

I. Subfamily THIOCAPSACEÆ, cells divide in three planes.

Genus :

1. *Thiocystis*.
2. *Thiocapsa*.
3. *Thiosarcina*.

II. Subfamily LAMPROCYSTACEÆ, cells divide first in three, then in two planes.

Genus :

1. *Lamprocystis*.

III. Subfamily THIOPEDIACEÆ, cells divide in two planes.

Genus :

1. *Thiopedia*.

IV. Subfamily AMEBOBACTERIACEÆ, cells divide in one plane.

Genus :

1. *Amebabacter*.
2. *Thiothece*.
3. *Thiodictyon*.
4. *Thiopolycoccus*.

V. Subfamily CHROMATIACEÆ.

Genus :

1. *Chromatium*.
2. *Rhabdochromatium*.
3. *Thiospirillum*.

CHAPTER V.

STERILIZATION.

STERILIZATION is the process of killing micro-organisms. A body is said to be sterile when all the bacteria in or upon it have been killed or removed. Sterilization is effected by the application of heat, by treating with certain chemicals, or, in the case of fluids, by filtration.

When spores are not present, bacteria are killed by exposure for twenty minutes to boiling water or steam. To destroy spores, however, a much longer exposure is necessary, in some cases several hours. Steam under a pressure of thirty pounds will give a temperature of 120° C.; this will kill all organisms and spores in fifteen minutes.

Dry heat is not so fatal to either bacteria or their spores as is moist heat or steam; therefore to sterilize by dry heat a higher temperature, sustained for a longer time, is necessary; 150° C. continued for one hour will kill all ordinary bacteria and their spores. Liquids and objects liable to be injured by dry heat can be sterilized only by steam.

The method of *discontinuous sterilization* is employed in sterilizing objects which would be injured by long exposure to a high temperature. The objects to be sterilized are first subjected to a temperature sufficiently high to destroy all bacteria not

in the spore condition, say 100° C. for twenty minutes. They are then allowed to cool for twenty-four hours, and again sterilized as before. This is repeated several times. The intermissions allow such spores as are present to develop into rods, and these are killed by the subsequent heating.

Pasteurization is the term applied to the partial sterilization of milk, effected by subjecting it to a temperature sufficiently high to kill all pathogenic and most of the souring and spore-forming bacteria, but not high enough to produce any physical changes, such as are brought about when the milk is sterilized. A temperature of 60° C. continued for fifteen or twenty minutes is usually sufficient, though temperatures as high as 85° C. are frequently employed. Enhanced keeping qualities and the destruction of pathogenic organisms are the results of pasteurization.

The chemical substances most frequently employed for sterilization or disinfection¹ are solutions of corrosive sublimate (1 : 1000), carbolic acid (1 : 20), or formalin (1 : 20). A substance that prevents the development of bacteria but does not destroy them is an antiseptic. One that destroys all germs and spores is a germicide or disinfectant. There are many gases, acids, salts, etc., that have antiseptic or germicidal properties.

Bacteria may be removed from a liquid by passing it through a properly constructed filter of un-

¹ Disinfection is the term applied to sterilization by means of chemicals.

glazed porcelain. The most satisfactory filters in use at present are the Chamberland and the Berkefeld.

FIG. 20. — Filter: *a*, porcelain bougie; *b*, attachment for suction-pump; *c*, reservoir; *d*, sterile receiver; *e*, rubber tube wired to bougie and reservoir.

FIG. 21. — Filter: *a*, porcelain bougie; *b*, attachment for suction-pump; *c*, reservoir; *d*, sterile receiver; *e*, perforated rubber collar; *f*, glass chimney for drawing the fluid around the bougie (Page).

Bacteria in the air are unable to pass through a cotton-wool filter, and consequently sterile flasks or

test-tubes stopped with a cotton-wool plug remain sterile indefinitely.

I. Steam Sterilization.

For objects liable to be injured by dry heat, such as culture-media, fluids, instruments, etc.:

FIG. 22.—Kitasato's filter: *a*, porcelain bougie; *b*, attachment for suction-pump; *c*, reservoir; *d*, sterile receiver.

(*a*) Fill the water-tank of the sterilizer¹ and start the flame.

(*b*) When the chamber is filled with steam place the objects to be sterilized within and close the door.

(*c*) If the objects will not be injured by prolonged heating, allow them to remain for one hour.

(*d*) If prolonged heating is injurious, allow them to remain twenty minutes, and repeat the process three times at intervals of twenty-four hours.

II. Steam Sterilization under Pressure.

For rapid and effective sterilization. In this method an "autoclave" is used.

(*a*) Adjust the safety-valve at the desired pressure, say thirty pounds.

See that an abundance of water is present, so that the steam will not be superheated.

¹ An "Arnold" sterilizer is the form usually employed.

(*b*) Place the objects to be sterilized in the chamber.

(*c*) Close the door and turn on the steam, first allowing the air present in the chamber to escape.

(*d*) Bring the temperature to, say 120° C. ; allow it to remain there fifteen minutes.

FIG. 23.—Arnold steam sterilizer, Boston Board of Health pattern.

(*e*) Shut off the steam and allow the apparatus to cool well below 100° C. before opening the door or allowing the steam to blow off.

III. Hot-air Sterilization.

For glassware and other objects not liable to be

injured by dry heat. The oven of an ordinary gas-stove into which a thermometer can be inserted makes an excellent hot-air sterilizer.

(a) Wash thoroughly, using, if necessary, the cleaning mixture recommended on page 14, all

FIGS. 24, 25.—Autoclaves for rapid sterilization by steam under pressure.

flasks, test-tubes, Petri dishes, etc., to be used in the preparation of culture-media.

(b) Plug the flasks and test-tubes with cotton.¹

¹ "Sliver" obtained from cotton-mills is excellent for plugging test-tubes, etc.

In the case of the test-tubes, the plugs should be tight enough so that the tubes can be lifted by them.

(c) Place in the hot-air sterilizer, close the door, and bring the temperature to 150° C.; keep it there

FIG. 26.—Hot-air sterilizer.

for three-quarters of an hour, or until the cotton plugs begin to turn brown.

(d) Allow the oven to cool before opening the door. Place the glassware in a clean place free from dust until used.

CHAPTER VI.

PREPARATION OF CULTURE-MEDIA.¹

I. Bouillon.	II. Gelatin.	III. Agar.
<p>1. Infuse finely chopped lean beef for twenty hours with <i>twice</i> its weight of distilled water in the refrigerator, say 1000 grams of meat, 2000 grams of water.</p> <p>2. Make up weight of meat-infusion (and meat) to original weight by adding water—<i>i. e.</i>, to 3000 grams.</p> <p>3. Filter infusion through cloth to remove meat.</p> <p>4. Weigh infusion, say 1800 grams.</p> <p>5. Set infusion on water-bath, keeping temperature below 60° C.</p> <p>6. Add peptone, 1 per cent., 18 grams; salt, 0.5 per cent., 9 grams.</p> <p>7. After ingredients are dissolved, titrate;² reaction probably + 2.3 to + 2.5 per cent.</p> <p>8. Neutralize (Fuller's method).³</p>	<p>Ditto.</p> <p>Ditto.</p> <p>Ditto.</p> <p>Ditto.</p> <p>Ditto.</p> <p>Ditto, and sheet gelatin, 10 per cent., 180 grams.</p> <p>Ditto, probably + 4.0 to + 5.0 per cent.</p> <p>Ditto.</p>	<p>Boil 30 grams of thread agar in 1 liter of water for half an hour. Make up loss by evaporation to a weight of 1000 grams.</p> <p>1. Infuse finely chopped lean beef for twenty hours with <i>its own</i> weight of distilled water in the refrigerator, say 1000 grams of meat, 1000 grams of water.</p> <p>2. Make up weight of meat-infusion (and meat) to original weight by adding water—<i>i. e.</i>, to 2000 grams.</p> <p>3. Ditto.</p> <p>4. Ditto, say 900 grams.</p> <p>5. Ditto.</p> <p>6. Add peptone, 2 per cent., 18 grams; salt, 1 per cent., 9 grams.</p> <p>7. Ditto, probably + 4.5 to + 4.7 per cent.</p> <p>8. Ditto.</p> <p>To the 900 grams of meat-infusion (containing now peptone and salt) add 900 grams of the 3 per cent. agar-jelly described at the head of this column.</p>

For notes see page 51.

¹ Substantially as recommended by the Bacteriologic Committee of the American Public Health Association. Some minor changes, suggested by Dr. H. W. Hill, in the *Report of the Health Department of Boston for 1898*, have been incorporated without detracting from their value as standard media.

² Acid media are denoted by the *plus* sign, and alkaline media by the *minus* sign; the degree of acidity or alkalinity is denoted by parts per hundred. Thus, a medium marked + 1.5 indicates that the medium is acid, and that 1.5 per cent. of $\frac{n}{1}$ sodium hydroxid is required to make it neutral to phenolphthalein.

³ Following is Fuller's method of obtaining the degree of reaction of culture-media:

- (a) Prepare a $\frac{n}{20}$ solution of sodium hydroxid.
- (b) Prepare a $\frac{n}{20}$ solution of hydrochloric acid.
- (c) Transfer 5 c.c. of the medium to be tested to a porcelain evaporating-dish.
- (d) Add 45 c.c. of distilled water.
- (e) Boil for three minutes.
- (f) Add 1 c.c. of a 0.5 per cent. solution of commercial phenolphthalein in 50 per cent. alcohol.
- (g) Titrate while still hot with the acid or alkali as required, and determine the reaction.

To determine exactly when the neutral point is reached, notice that in bright daylight the first change that can be seen on the addition of alkali is a very faint darkening of the fluid, which on the addition of more alkali becomes a more evident color and develops into what may be described as an Italian pink. A still further addition of alkali suddenly develops a clear and bright pink color, and this is the reaction always to be obtained.

(h) When the reaction has been obtained, calculate the amount necessary to neutralize the bulk of the medium or to produce the required reaction, and add the proper amount of a normal solution of the acid or alkali.

9. Heat over boiling water (or steam) bath thirty minutes.

10. Restore weight lost by evaporation to original weight of filtered meat-infusion, for bouillon and gelatin, and to *twice* that weight for agar—1800 grams in *each case*.

11. Titrate, reaction probably $+ 0.3$ to $+ 0.5$ per cent.

12. Adjust reaction to final point desired, $+ 1.5$ per cent.

FIG. 27.—Filter for culture-media.

13. Boil five minutes over free flame, with stirring.

14. Add water if necessary to make up loss by evaporation to 1800 grams.

15. Filter through absorbent cotton, passing the filtrate through the filter repeatedly until clear.

16. Titrate to determine whether or not the desired reaction has been maintained.

17. Tube and sterilize for fifteen minutes in the steam sterilizer on three successive days.¹

Some of the gelatin- and agar-tubes after the

FIG. 28.—Funnel for filling tubes with culture-media : *a*, funnel containing the culture-media in liquid condition ; *b*, pinch-cock by which the flow of fluid into the test-tube is regulated ; *c*, rubber tubing (Warren).

¹ Allow the chamber to fill with steam before placing the media within. Do not leave the media in the sterilizer to cool. Plunge gelatin-tubes into ice-water immediately after each sterilization, in order to maintain a high melting-point.

FIG. 29.—Providence Health Department tube of heavy glass, with etched surface for writing data. These tubes are etched by dipping for thirty seconds in "white acid."

FIG. 30.—Potato-tube (Mallory and Wright).

last sterilization should be allowed to solidify with slanting surfaces.

IV. Media from Meat-extracts.

(a) Mix thoroughly the white of 1 egg with 1000 c.c. of water for bouillon or gelatin; with 500 c.c. of water for agar.

(b) Proceed then as directed in the above table, beginning with No. 4, substituting "white of egg solution" for "infusion." At No. 6 add 0.5 per cent. of Liebig's extract of beef for bouillon or gelatin, 1.0 per cent. for agar.

V. Potato.

(a) Select large sound potatoes and wash thoroughly.

(b) Cut off the ends, and with a sterile cork-borer cut out cylinders of the potato a little smaller than the tubes in which they are to be placed. Handle the potatoes under water as much as possible, to prevent darkening of the surface.

(c) Cut the cylinders into two equal parts by a diagonal cut.

(d) Place in cold running water for twelve to eighteen hours. This will usually render the potato neutral.

(If necessary to change the reaction of the potato, steam in a measured quantity of distilled water for one-half hour. Titrate and add the required amount of $\frac{n}{I}$ sodium hydroxid, and repeat the boiling for thirty minutes.)

(e) Distribute in tubes in the bottom of which a small amount of non-absorbent cotton or a short piece of glass rod has been placed, and sterilize in

the steam sterilizer for thirty minutes on three successive days.

VI. Dextrose, Lactose, and Saccharose Bouillon.

(a) After filtration of the meat-infusion, prepared as above described, place in an Erlenmeyer flask, and inoculate with a fluid culture of the *Bacillus coli* or an allied gas-producer.

(b) Place in the incubator at 37.5° for twenty-four hours.

FIG. 31.—The Smith fermentation-tube.

FIG. 32.—A graduated fermentation-tube.

This removes the meat-sugar.

(c) From this infusion prepare bouillon in the ordinary way.

(d) To the completed broth add 1 per cent. of the required sugar.

(e) Distribute in test-tubes or in fermentation-tubes,¹ and sterilize in the steam sterilizer on three successive days.

¹ A small test-tube inverted inside a large one will answer for a fermentation-tube.

VII. Milk and Litmus Milk.

(a) Heat fresh milk for fifteen minutes in the steam sterilizer.

(b) Place in the ice-box over night.

(c) Siphon off the milk, without cream or sediment.

FIG. 33.—The Hill fermentation-tube.

(d) Titrate.

(e) If less than 2 per cent. acid to phenolphthalein, place in tubes and sterilize for twenty minutes on four successive days in the steam sterilizer. If more than 2 per cent. acid, adjust to + 1.5 per cent.

by the addition of $\frac{n}{I}$ sodium hydroxid.

(f) A solution of litmus may be added just

FIG. 34.—Siphon, with one-way valve for starting the flow of serum. The end of the glass tube is turned over to prevent the clot from entering.

FIG. 35.—Coagulator for blood-serum tubes. Providence Health Department pattern, with wooden rack for holding tubes away from sides. When in use, the four sides and the space below are covered with asbestos boards.

previous to its distribution in tubes, sufficient to give the milk a pale-blue color.

VIII. Blood-serum (*Löffler's*).

(a) Receive freshly drawn beef blood in sterile jars.

(b) Allow twenty minutes for coagulation to begin, then with a sterile glass-rod break up any adhesions between the coagulum and the jar.

(c) Allow the jars to stand twenty-four hours in the ice-box.

(d) Siphon off the clear serum.¹

(e) To 3 parts of serum add 1 part of 1 per cent. dextrose bouillon.²

(f) Adjust to + 0.8 per cent.

(g) Distribute in tubes and solidify with a slanting surface by heating for three hours in a blood-serum coagulator.

(h) Sterilize in the steam sterilizer for twenty minutes on three successive days.

¹ If the serum is not clear, filter through the coagulum left after the filtration of bouillon, as suggested by Hill.

² If 1.25 per cent. glycerin is added also, it seems to prevent the tubes from drying.

CHAPTER VII.

CULTURES OF BACTERIA.

OWING to their small size and to their similarity of form, the different species of bacteria cannot be recognized by microscopic examination alone. Recourse must be had to a study of their biologic peculiarities. For this purpose it is necessary to grow them on artificial culture-media. If a single germ is planted on a suitable nutrient medium, and is protected from contamination, it multiplies rapidly and forms a colony. Such a colony is composed of but one species, since all its members are the descendants of a single germ. Cultures made from such a colony are known as "pure cultures," since they contain but one species. From a study of the behavior of these pure cultures, under different conditions, the diagnostic characters of the species are determined.

I. BOUILLON CULTURES.

1. Inoculate a tube of bouillon by touching the culture to be studied with a sterile platinum needle, and then dipping the needle in the bouillon.

2. Place at the room-temperature or in the incubator, and examine from day to day, and note any changes as follows :

(a) Does the bouillon become uniformly clouded, or only at the surface or bottom?

(b) Does a pellicle form on the surface or a deposit at the bottom?

FIG. 36.—Method of holding tubes during inoculation.

(c) Is the color, odor, or reaction of the bouillon changed?

II. GELATIN OR AGAR CULTURES.

I. "Stab" Cultures.¹

(1) Sterilize a straight platinum needle and touch it to the culture to be studied; hold the gelatin- or agar-tube upside down, remove the cotton plug, and stab the needle carefully up through the center of the medium nearly to the bottom of the

¹ "Stab" cultures are usually made in gelatin-tubes; "stroke" cultures, on agar-tubes.

tube ; withdraw the needle carefully and replace the cotton plug.

(2) Allow to grow at the room-temperature, if gelatin is used ; at either room- or incubator-temperature, if agar is used ; examine from day to day.

(3) Note in stab cultures :

(a) Does the growth appear along the line of puncture, at the surface, or in both places ?

(b) Is the surface growth abundant or scanty ; does it spread over the whole surface or is it confined to the point of puncture ; is its margin regular or indented ; is it flat or raised, dry or moist ; what is its color, luster ?

(c) Is the medium changed in color, odor, or condition ?

(d) In gelatin cultures, if liquefaction takes place, is it at the surface, deep, or throughout the line of puncture ? What is the

form of the liquefied area.

The following terms have been suggested by

FIG. 37.—*a*, "Stab" culture ; *b*, "stroke" culture.

FIG. 38.—Various forms of gelatin "stab" cultures: *a*, *Bacillus typhosus*; *b*, *Bacterium anthracis*; *c*, *Bacillus mycoides*; *d*, *Bacillus mesentericus*; *e*, *Bacillus oedematis*; *f*, *Bacillus radiatus*.

Chester¹ for describing the characters of gelatin stab cultures:

I. Non-liquefying Cultures.

Line of puncture may be—

- Filiform*, uniform growth without special characters.
- Nodose*, consisting of closely aggregated colonies.
- Beaded*, consisting of loosely placed, disjointed colonies.
- Papillate*, beset with papillate extensions.
- Echinate*, beset with acicular extensions.
- Villous*, beset with short, undivided, hair-like extensions.
- Plumose*, a delicate feathery growth.
- Arborescent*, branched or tree-like, beset with branched hair-like extensions.

¹ *Eleventh Annual Report of the Delaware College Agricultural Experiment Station*, Newark, Delaware, 1898-1899.

II. Liquefying Cultures.

Line of liquefaction may be—

Crateriform, a saucer-shaped liquefaction.

Saccate, shape of an elongated sac, tubular, cylindrical.

Infundibuliform, shape of a funnel, conical.

Napiform, shape of a turnip.

Fusiform, shape of a parsnip, narrow at either end, broadest below the surface.

Stratiform, liquefaction extending to walls of the tube and downward horizontally.

FIG. 39.—*Microspira comma* (Asiatic cholera): gelatin puncture cultures aged forty-eight and sixty hours (Shakespeare).

2. "Stroke" Cultures.

(1) Sterilize the platinum needle, touch it to the

FIG. 40.—*Microspira Finkleri*: gelatin puncture cultures aged forty-eight and sixty hours (Shakespeare).

FIG. 41.—*Microspira Metschnikovi*: puncture culture in gelatin forty-eight hours old (Fränkel and Pfeiffer).

culture to be studied, and draw it over the surface of an agar- or gelatin-tube that has been solidified with a slanting surface.

(2) Allow to grow as before, and examine from day to day.

(3) Note:

(a) What are the size and shape of the streak?

(b) What is the character of the margin?

(c) Is the growth abundant or scanty, flat or raised, opaque or transparent?¹

(d) What is the color, luster?

(e) Has the color, consistence, or odor of the medium changed?

III. POTATO AND BLOOD-SERUM CULTURES.

Stroke cultures are employed in using potato or blood-serum tubes. The methods of study are essentially the same as described for gelatin or agar cultures.

IV. PLATE CULTURES.

In making plate cultures the nutrient medium is liquefied and a very small portion of the culture to be studied mixed with it; it is then poured into sterile Petri dishes, solidified, and the colonies allowed to develop. By this method the bacteria are scattered through the medium, and the colonies that develop are the descendants of a single germ. This permits different species to be separated from a mixture in pure culture, and the peculiarities of

¹ For descriptive terms, see p. 70, *et seq.*

“colonies” in distinction from “cultures” to be determined.

1. Liquefy three gelatin- or agar-tubes¹ by placing in a water-bath. Gelatin-tubes must not be heated above 40° C. Agar-tubes may be heated to 100° C., but must be cooled to 40° C. before the inoculations are made.

2. With a sterile platinum needle transfer a

FIG. 42.—Petri dish with colonies.

minute portion of a culture to the liquefied medium in the first tube.

3. Shake the tube thoroughly without producing bubbles in the medium, and transfer a loopful to the second liquefied tube.

4. Shake the second tube, and transfer a loopful to the third tube.

¹ Bouillon-tubes or tubes of sterile water may be substituted for the first two gelatin- or agar-tubes when it is desired to make but one plate.

FIG. 43.—Brown University electric water or paraffin bath.¹

¹ A porcelain-lined dish fitted with a tin cover, provided with four doors. In the figure the cover is raised from the dish to show the thirty candle-power electric lamp which projects through the cover into the water or paraffin below. The lamp is provided with a regulating socket giving

5. Shake the third tube, and pour the contents of the three tubes into sterile Petri dishes, having first passed the lip of each tube through the flame.

6. On the surface of the first Petri dish, before

FIG. 44.—The various appearances of colonies of bacteria under the microscope: *a*, colony of *Bacterium parvum*; *b*, colony of *Bacillus polypiformis*; *c*, colony of *Bacillus radiatus*.

the medium has entirely solidified, press a sterile¹ cover-glass or small piece of mica.

candle-powers from five to thirty. By adjusting the socket to the different candle-powers the requisite temperature of water or paraffin is secured and indicated by the thermometer. When used as a water-bath, the dish is provided with a wire rack to support the tubes to be melted. When used for paraffin, little baskets of wire gauze, containing the specimens to be embedded, are hung about the sides. It is convenient to have the water-bath kept at a temperature of 40° C. In this gelatin-tubes may be melted, or agar-tubes, after being melted at a higher temperature, may be cooled down and kept melted until used.

¹ Sterilize by heating in the flame. Allow it to cool before placing on the plate.

7. Allow to develop as directed for tube cultures, and examine from day to day, or oftener as necessary.

8. In plate cultures note :

(a) Is there a difference in the number of colonies in the three dishes? The use of the three tubes and plates is for the purpose of reducing the number of colonies, so that only a few will be present in the third plate.

(b) Is there more than one kind of colony present?

Notice that colonies on the surface differ from those below the surface, though of the same species. If the culture from which the inoculations were made is a pure culture, and if no germs have been allowed to enter during the process of making the plates, there should be but one kind of colony present.

(c) What are the size, shape, texture, and color of the colonies, both surface and deep? What is the character of their margins?

The following terms are those suggested by Chester for the description of colonies :

I. Form.

Punctiform, dimensions too small for definition.

Round, of a more or less circular outline.

Irregular.

Elliptical.

Fusiform, spindle-shaped.

Cochleate, spiral or twisted like a shell.

Ameboid, very irregular, streaming.

Mycelioid, a filamentous colony with the radiate character of a mould.

Filamentous, an irregular mass of loosely woven filaments.

Floccose, of a dense woolly structure.

Rhizoid, of an irregular, branched root-like character.

Conglomerate, an aggregate of colonies of similar size and form.

Toruloid, an aggregate of colonies like the budding of the yeast plant.

Rosulate, like a rosette.

II. Surface Elevation.

1. General character of a surface as a whole.

Flat, thin, leafy, spreading over the surface.

Effused, spread over the surface as a thin, veily layer more delicate than the preceding.

Raised, growth thick with abrupt terraced edges.

Convex, surface the segment of a circle, but very flatly convex.

Pulvinate, surface the segment of a circle, but decidedly convex.

Capitate, surface semi-spherical.

2. Detailed characters of surface.

Smooth, surface even, without any of the following distinctive characters.

Alveolate, honeycombed.

Punctate, dotted with punctures.

Bullate, blistered.

Vesicular, more or less covered with mi-

nute vesicles due to gas formation ;
more minute than bullate.

Verrucous, bearing wart-like prominences.

Squamose, scaly.

Echinate, beset with pointed prominences.

Papillate, beset with nipple-like processes.

Rugose, short irregular folds due to shrinkage.

Corrugated, in long folds due to shrinkage.

Contoured, a smoothly undulating surface.

Rimose, abounding in clefts or cracks.

III. Microscopic Structure.

1. Refraction weak. Outline and surface of relief not strongly defined.

2. Refraction strong. Outline and surface of relief strongly marked.

(a) Dense, not filamentous colonies.

1. General.

Amorphous, without definite structure.

Hyaline, clear and colorless.

Homogeneous, structure uniform throughout all parts of the colony.

Homochromous, colony of uniform color throughout.

2. Granulations or blotchings.

Finely granular.

Coarsely granular.

Grumous, coarser than preceding.

Moruloid, segmented.

Clouded, having a pale ground with ill-defined patches of a deeper tint.

3. Colony marking or striping.

Reticulate, in the form of a network.*Areolate*, divided into rather irregular or angular spaces.*Gyrose*, marked by wavy lines.*Marmorated*, traversed by vein-like markings.*Rivulose*, marked by lines like the rivers on a map.*Rimose*, showing cracks or clefts.

(b) Filamentous colonies.

Filamentous, as already defined.*Floccose*, composed of filaments densely packed.*Curled*, filaments in parallel strands.

IV. Edges of Colonies.

Entire, without toothing or division.*Undulate*, wavy.*Repand*, like the border of an open umbrella.*Erose*, as if gnawed, irregularly toothed.*Lobate*, divided into lobes.*Lobulate*, minutely lobate.*Auriculate*, with ear-like lobes.*Lacerate*, irregularly cleft, as if torn.*Fimbriate*, fringed.*Ciliate*, hair-like extensions.

<i>Tufted,</i>	}	as already defined.
<i>Filamentous,</i>		
<i>Floccose,</i>		
<i>Curled,</i>		

V Optical Characters.

Transparent.

Vitreous, transparent and colorless.

Oleaginous, transparent and yellow.

Resinous, transparent and brown.

Translucent.

FIGS. 45, 46.—The various appearances of colonies of bacteria under the microscope: *a*, colony of *Bacillus muscoides* (Liborius); *b*, colony of *Bacterium anthracis* (Flügge).

Porcelaneous, translucent and white.

Opalescent, translucent, grayish white by reflected light, smoky brown by transmitted light.

Nacreous, translucent, grayish white with pearly luster.

Sebaceous, translucent, yellowish or grayish white.

Butyrous, translucent and yellow.

Ceraceous, translucent and wax-colored.

Opaque.

Cretaceous, opaque and white ; chalky.

Dull, without luster.

Glistening.

Fluorescent.

Iridescent.

Phosphorescent.

(*d*) Is there growth beneath the cover-glass or mica plate? This determines, roughly, whether they require oxygen for their growth or not (aërobic or anaërobic).

(*e*) In the gelatin plates is the gelatin liquefied, and what is the nature of the liquefaction?

V. IMPRESSION OR ADHESIVE PREPARATIONS OF COLONIES.

An entire colony of bacteria may be preserved as a microscopic specimen.

(*a*) Slightly warm a clean cover-glass.

(*b*) Lay it upon the surface of the gelatin or agar containing the colonies. Apply sufficient pressure to remove all air-bubbles, but not enough to disturb the colony.

(*c*) Remove the cover, gently lifting it from one side. The colonies will adhere to the glass.

(*d*) Dry, fix, stain, and mount as for ordinary preparations.

Museum preparations of gelatin or agar tube or plate cultures may be made by exposing the cultures to formaldehyd vapor until the growth is killed, and then sealing the tubes or plates tightly with sealing-wax or paraffin.

FIG. 47.—*Bacterium tuberculosis*: adhesive preparation from a fourteen-day blood-serum culture; $\times 100$ (Fränkel and Pfeiffer).

VI. CULTURES IN THE FERMENTATION-TUBE.

This method is for the purpose of studying gas formation, and for the study of the aërobic or anaërobic properties of organisms.

(*a*) Prepare fermentation-tubes with dextrose, lactose, or saccharose bouillon.

(*b*) Inoculate the tubes by floating a little of the

culture to be studied on the fluid in the bulb with a sterile needle.

(c) In the case of gas formation, at the end of every twenty-four hours, for several days, mark the level of the fluid in the closed branch upon the tube or measure it with a millimeter scale.

(d) Record the result in percentages of the length

FIG. 48.—*Bacterium anthracis*: colony three days old upon a gelatin plate; adhesive preparation; $\times 1000$ (Fränkel and Pfeiffer).

of the closed branch. If 1 cm. of gas forms in a 10 cm. tube, 10 per cent. of gas is said to have formed.

(e) To test the relative amount of carbon dioxide and hydrogen present. Fill the bulb completely with a 2 per cent. solution of sodium hydroxid. Place the thumb over the mouth of the bulb, and

run the mixture six or eight times through the length of the tube, returning the remaining gas to the closed branch before removing the thumb. Measure the amount of gas remaining; the difference between this and the former measurement shows in millimeters the amount of carbon dioxid absorbed by the alkali. The remaining gas, mostly hydrogen, may be transferred to the bulb and exploded by a flame. The proportion of hydrogen to carbon dioxid is usually expressed in the form of a fraction called the gas formula, $\frac{H}{CO_2}$.

The fermentation-tube affords a ready method of determining the oxygen requirement of bacteria. Growth, indicated by cloudiness, in the bulb only, is to be found only among obligatory aërobes; in the closed branch only, among obligatory anaërobes; while growth in both, only among the facultative anaërobes.

VII. ANAËROBIC CULTURES.

Growth under the mica plate or cover-glass¹ and in the fermentation-tube are methods for the determination of the aërobic properties of organisms. For the growth of strictly anaërobic forms special methods have been devised:

1. Place the cultures in a vessel from which the air can be withdrawn and hydrogen substituted.

2. **Buchner's Method.**—Use two test-tubes, one inside the other. The outer one is partially filled

¹ See page 75.

with pyrogallic acid made alkaline with sodium hydrate, and is sealed tightly with a rubber stopper. The inner tube contains the culture. The oxygen is absorbed by the mixture in the outer tube.

3. Wright's Method.—Make an ordinary culture in a test-tube. Clip off any superfluous cotton from the plug, and push the plug into the tube so

FIG. 49.—Novy's jars for anaërobic cultures.

that it lies 1 centimeter below the mouth. For test-tubes $6 \times \frac{3}{4}$ inches, run into the cotton plug, from a pipet, approximately $\frac{1}{2}$ c.c. of a freshly prepared solution of pyrogallic acid (1 part of acid, 1 part of water), and then approximately 1 c.c. of a solution of sodium hydrate (1 part of sodium hydrate, 2 parts of water). Quickly insert a rubber stopper into the tube.

4. Make a hanging-drop culture. On one side of the cover-glass introduce a little pyrogallic acid, and on the other side a little sodium hydrate, so that it runs around and unites with the acid. Seal with vaselin.

5. Distribute the germs to be studied in bouillon or in liquefied gelatin or agar, and draw some of the solution into sterile pieces of glass tubing of small caliber. When the tube is full seal the ends in a flame.

6. Put large quantities of culture-medium in the tubes and puncture deeply. The surface of the medium is then covered with sterile oil.

7. **Park's Method.**—Cover the culture-medium with melted paraffin.¹ Sterilize by the ordinary methods, and when cool enough for inoculation, but before the paraffin solidifies, inoculate through the paraffin into the medium below.

Wright recommends² the following precautions in growing anaërobic bacteria :

1. The medium should contain 1 per cent. of glucose, and should be boiled and cooled immediately before inoculation.

2. The medium should be freshly prepared.

3. The reaction should not be more acid to phenolphthalein than +1.5. With 1 per cent. glucose bouillon, growth is better if the reaction is nearer the neutral point of phenolphthalein than +1.5.

VIII. DEMONSTRATION OF LIQUEFYING FERMENT.

(a) Inoculate several gelatin-tubes with *Bacillus prodigiosus*.

(b) Allow them to grow until all the gelatin is liquefied.

¹ Melting point, 42° C.

² *Jour. Boston. Soc. Med. Sci.*, vol. v., No. 4, p. 114.

(c) Add $\frac{1}{2}$ c.c. of chloroform, or 5 per cent. carbolic acid, to each tube, shake thoroughly, and filter.

(d) Add the filtrate, now containing no living bacteria, to tubes of sterile gelatin. Note the liquefaction that takes place, caused by the ferment produced by the bacteria in the first set of tubes.

IX. ISOLATION OF SPECIES.

Given a bouillon culture, containing three species of bacteria, to isolate the species.¹

(a) Liquefy three gelatin or agar tubes and number 1, 2, and 3 respectively.²

(b) Transfer a minute loopful of the bouillon culture to tube No. 1.

(c) Shake thoroughly, and transfer 2 loopfuls from tube No. 1 to tube No. 2.

(a) Shake and transfer 3 loopfuls from tube No. 2 to tube No. 3.

(e) Flame the lips of the tubes and pour their contents into sterile Petri dishes.

(f) Examine in twelve to twenty-four hours.

(g) Select the dish in which the colonies are well developed, and in which they have not run together; look for three kinds of colonies.

If more than three kinds are present, it shows

¹ This method is applicable to the separation of species from any fluid.

² Tubes of bouillon or sterile water may be substituted for tubes Nos. 1 and 2, in which case, of course, but one plate can be made—*i. e.*, from tube No. 3.

that others have been allowed to enter through carelessness in manipulation.

(h) Record the appearance of the different colonies, and inoculate them as pure cultures in tubes of culture-media. Study them as directed in the following chapter.

CHAPTER VIII.

DETERMINATION OF SPECIES.

I. MORPHOLOGY AND LIFE-HISTORY OF A SPECIES.

THE following points in the morphology and life-history of any form of bacterium must be known before it can be fully described or assigned a place in any particular species.¹

I. SOURCE AND HABITAT.

II. MORPHOLOGIC CHARACTERS.

1. Form.
2. Dimensions.
3. Manner of grouping and arrangement in the growths.
4. Staining powers: (*a*) with aqueous stains; (*b*) by Gram's method.
5. Presence or absence of capsule.
6. Presence or absence of flagella (motility).
7. Spore-formation.
8. Tendency to pleomorphism.
9. Involution and degeneration forms.

III. BIOLOGIC CHARACTERS.

A. Cultural characteristics, mode of growth in and upon—

1. Bouillon.
2. Gelatin plates (single colonies, surface and deep).
3. Gelatin-tubes.
4. Agar plates (single colonies, surface and deep).

¹ These are the points recommended by the Committee of Bacteriologists of the American Public Health Association.

5. Agar-tubes.
6. Potato.
7. Milk.
8. Blood-serum.

B. Biochemic features.

1. Temperature relationship (activity of growth at 18°-22° C. and at 36°-38° C. and thermal death-point).
2. Relation to free oxygen (aërobic and anaërobic growth).
3. Relation of growth to acidity and alkalinity of media.
4. Action upon gelatin (presence or absence of liquefaction).
5. Action upon proteids (milk and serum).
6. Action upon carbohydrates (fermentation and gas formation).
7. Action upon nitrates.
8. Production of indol.
9. Production of acid or alkali.
10. Pigment formation.
11. Development of odor.

C. Pathogenesis.

The following tests are of value in certain cases :

I. MORPHOLOGIC.

1. Staining reactions with special stains.
2. Study of flagella by special stains.
3. Permanency of morphologic characters after long-continued growth and successive transplantation upon artificial media.

4. Photographic reproductions of isolated bacteria.
5. Cover-glass impressions.

II. PHYSIOLOGIC.

A. Cultural characteristics, mode of growth in or upon—

1. Litmus gelatin.
2. Löffler's blood-serum.
3. Synthesized media.
4. Photographic reproduction of characteristic cultures.

STANDARD CHART FOR BACTERIAL ANALYSIS.

NAME	SOURCE	HABITAT	DATE	REPORTED BY
<p><i>Form and arrangement</i> in bouillon, grown hours at 18°-20° C.; ditto, grown hours at 36°-38° C.</p> <p><i>Micrococci</i>, single, pairs, chains, tetrads, or cubical packets; <i>Bacillus</i>, single, pairs, chains, or filaments; <i>Spirillum</i>, comma, spiral.</p> <p><i>Size</i>, length μ, breadth μ; extreme lengths from μ to μ.</p> <p><i>Capsules</i>, none observed, easily observed or demonstrated. Conditions under which they are present, agar, serum, milk, or spores, none observed within hours at ° C. on method. When present are polar, central, cells swollen. Are killed at 100° C. in minutes.</p> <p>Germinate within hours at ° C. Stain by method. or when stained with ° C. for hours.</p> <p><i>Vacuoles</i>, observed when grown on at ° C., or more pronounced in cultures grown at ° C. for hours.</p> <p><i>Motility</i>, sluggish or active, rotary or direct, more pronounced in method; are monotrichous, lophotrichous, amphitrichous, peritrichous.</p> <p>Flagella stain by ° C. for days.</p> <p><i>Pleomorphicism</i>, observed in cultures grown at ° C. for days.</p> <p><i>Stain</i>, easily or with difficulty with , uniformly or irregularly. Stained or decolorized by Gram's method.</p>				
<p>GELATIN OR AGAR PLATES.</p> <p>Size.</p> <p>Shape.</p> <p>Margin.</p> <p>Textures.</p> <p>Color.</p> <p>Under mica plate.</p>		<p>Surface colonies.</p> <p>Gelatin.</p> <p>Agar.</p>		<p>Deep colonies.</p> <p>Gelatin.</p> <p>Agar.</p>
<p>GELATIN OR AGAR-TUBE.</p> <p><i>Puncture</i>.</p> <p>Form.</p> <p>Surface growth.</p> <p>Size.</p> <p>Shape.</p> <p>Margin.</p> <p>Texture.</p> <p>Color.</p> <p>Consistence.</p> <p>Deep growth.</p> <p>Gas-bubbles.</p> <p><i>Streak</i>.</p> <p>Size.</p> <p>Shape.</p> <p>Margin.</p> <p>Surface relief.</p> <p>Light transmission.</p> <p>Color.</p> <p>Luster.</p> <p>Consistence.</p> <p><i>Change in medium</i>.</p> <p>Color.</p> <p>Consistence.</p> <p>Odor.</p>		<p>Bouillon.</p> <p>Opacity begins after hrs. at ° C.</p> <p>Pellicle forms in hrs. at ° C.</p> <p>Color appears in ... hrs. at ° C.</p> <p>Thickness.</p> <p>Consistence.</p> <p>Deposit forms in hrs. at ° C.</p> <p>Amount.</p> <p>Color.</p> <p>Character, compact, flocculent, granular, flaky, or viscid on agitation.</p> <p>Color.</p> <p>Odor.</p> <p>Reaction, after hrs. at ° C.</p>		<p>POTATO OR BLOOD-SERUM.</p> <p>Size.</p> <p>Shape.</p> <p>Margin.</p> <p>Surface relief.</p> <p>Color.</p> <p>Luster.</p> <p>Texture.</p> <p>Consistence.</p> <p>Liquefaction.</p> <p>Gas production.</p>
<p>NITRATE BROTH.</p> <p>Reduction of nitrates.</p> <p>Dextrose-free bouillon.</p> <p>Indol production.</p>		<p>SKETCH OF GERM AND COLONY.</p>		
<p>MILK.</p> <p><i>Curd</i>, curdles or does not curdle in days at 18°-20° C. or 36°-38° C.</p> <p>" " " " on boiling after days at ° C.</p> <p>hard or soft, in one mass or in fragments, gas-bubbles.</p> <p>changed or unchanged by boiling.</p> <p><i>Whey</i>, separates from curd or not, amount, transparent or turbid.</p> <p><i>Reaction</i>, in days at ° C. Color.</p> <p><i>Digestion</i>, becomes gradually transparent without forming curds.</p> <p>complete in days at ° C.</p> <p>effects of boiling, odor, clear or cloudy, watery or viscid.</p> <p>reaction at end of digestion</p>		<p>SKETCH OF GERM AND COLONY.</p>		
<p>SUGAR BOUILLON IN FERMENTATION-TUBES</p> <p><i>Dextrose</i></p> <p><i>Lactose</i></p> <p><i>Saccharose</i></p>		<p>Amount of gas in ϕ formed at 36°-38° C.</p> <p>in 1 d., 2 ds., 3 ds., days.</p>		<p>Reaction of fluid in after days at ° C.</p>
<p>PIGMENT,</p> <p>developed in presence or absence of oxygen.</p> <p>" " " " in cultures at ° C. in hours.</p> <p>color, changed to by acid or alkali.</p> <p>soluble in, spectrum</p>		<p>CO₂ ϕ.</p> <p>H ϕ.</p> <p>Pellicle.</p> <p>Opacity.</p> <p>Color.</p>		
<p>PATHOGENESIS.</p>				
<p>OPTIMUM TEMPERATURE ° C.</p> <p>GROWTH LIMITS ° C. to ° C.</p> <p>THERMAL DEATH-POINT ° C., time of exposure minutes.</p>		<p>RELATION OF GROWTH TO ACIDITY OR ALKALINITY OF MEDIUM.</p> <p>..... ϕ acid to ϕ alkaline.</p>		
<p>PRODUCTION OF ACIDS OR ALKALIES.</p> <p>Carbohydrates absent or present.</p>		<p>RELATION TO FREE OXYGEN.</p> <p><i>Obligatory aërobie.</i></p> <p><i>Facultative anaërobie.</i></p> <p><i>Obligatory anaërobie.</i></p>		

B. Biochemic.

1. Minimum, optimum, and maximum temperatures of growth.

2. Growth in atmospheres of various inert gases (when anaërobic power of growth has been determined).

3. Optimum reaction of media and reaction limits, acid and alkaline (indicated by phenolphthalein).

4. Chemic properties and solubility of pigments produced, and spectroscopic observations upon the pigment.

C. Pathogenesis.

1. Inoculation of various species of animals, with minute study of the pathologic changes produced.

2. Immunity-producing properties.

3. Agglutinating properties of specific sera.

4. Determination and isolation of toxic substances (from non-pathogenic, as well as from pathogenic, bacteria).

A suitable blank should be prepared, on which are recorded the observations made on each species. The appended form (pages 86, 87) is substantially the one recommended by the Bacteriologic Committee of the American Public Health Association.

A further explanation in regard to some of these points is required.

(a) **Study of Form and Grouping.**—Determine and describe the morphology from the growth obtained upon at least one solid medium and in at least one liquid medium. Growth at 36°–38° C. should in general be not older than from twenty-four to forty-eight hours, while growth at room-temperature (18°–22° C.) should be not older than from forty-eight to seventy-two hours. Growth on solid media may be studied from cover-glass preparations; in liquid media growth is best observed

in the hanging-drop, preferably in a fresh medium inoculated with a very small amount of the culture to be examined. It is desirable that the form and grouping be determined in bouillon, gelatin, and on agar, and that any variation found upon the examination of the growth on other media be accurately noted.

(b) **Test for Motility.**—For the study of motility the hanging-drop preparations should be made from young cultures grown at or near the optimum temperature for only a few (six to eighteen) hours.

(c) **Tests for Spores.**—The tests for the presence of spores are :

(a) Do colonies develop from cultures which have been subjected to a temperature of 80° C. for ten minutes?

(b) Are there highly refracting bodies within the bacteria in unstained preparations, and can they be demonstrated by the spore-staining methods?

Cultures to be tested should be grown for forty-eight hours in bouillon, and when possible, at 36° – 38° C. Three loopfuls of this culture after agitation are transferred to tubes containing 10 c.c. of bouillon. This is exposed to a temperature of 80° C. for ten minutes, and then placed under conditions favorable for the development of any of the organisms which may have survived.

(d) **Pleomorphism.**—In regard to pleomorphism, attention is called to the variations in size and shape brought about by the following conditions of growth :

(a) At different temperatures.

(*b*) Upon or in media of different composition.

(*c*) Upon or in media of different degrees of acidity and alkalinity.

(*d*) In cultures of different ages.

(*e*) As well as to the variations in the size and shape of different individual bacteria obtained from one culture and appearing often in the same field of view—*i. e.*, subjected to exactly the same conditions of growth.

(*e*) **Determination of the Thermal Death-point.**—In determining the thermal death-point, the facts required to be known are (1) the time of exposure to heat, (2) the presence or absence of moisture, (3) the presence or absence of spores, (4) the age of the culture, (5) the amount of the culture used for the tests, and (6) the character of the containing vessel.

The temperature required to destroy the species under consideration is to be determined within 2 degrees C.; thus, if samples are exposed to temperatures of 50°, 52°, 54°, 56°, 58°, and 60° C., and it is found that development in a suitable medium occurs after exposure to 56° C., but not after exposure to 58° and 60° C., the thermal death-point is to be given as 58° C., although further study might show it to be somewhat less than this.

(*f*) **Relation to Free Oxygen.**—For the methods of determining the aërobic properties of organisms, see under Anaërobic Cultures, on page 78.

(*g*) **Relation to Acidity and Alkalinity of Media.**—In determining the relation of growth to

acidity and alkalinity of media, all that is necessary is to add to tubes containing equal quantities of any of the usual media a calculated amount of a standardized solution of hydrochloric acid or of sodium hydroxid to obtain the desired reaction. A record should be made of cultures upon at least one medium reacting +3 per cent., +1.5 per cent., neutral, and -1.5 per cent. to phenolphthalein.

(h) Action upon Carbohydrates.—For the methods of determining the action upon carbohydrates and for measuring the gas production, see under Cultures in the Fermentation-tube, page 76.

(i) Action upon Nitrates.—To determine the power of certain bacteria to reduce nitrates to nitrites and ammonia, incubate them for seven days at 20° C. in the following:

NITRATE BROTH.

Peptone,	1 gm.;
Potassium nitrate,	0.2 gm.;
Tap water, ¹	1000 c.c.

Submit the inoculated tubes and also several uninoculated control-tubes to the following tests for nitrites:

Prepare two solutions:

I. Naphthylamin,	0.1 gm.;	✓
Distilled water,	20 c.c.	

Boil until the naphthylamin is dissolved; cool,

¹ Better than distilled water because of the other salts present, which favor the growth of the bacteria.

filter, and add the filtrate to 150 c.c. of dilute (1 to 16) hydric acetate.

II. Sulphanilic acid, 0.5 c.c.;
Dilute (1 to 16) hydric acetate, 150 c.c.

Keep these solutions in separate glass bottles, tightly stoppered, and mix in equal parts before use.

To 3 c.c. of the solution to be tested, in a perfectly clean test-tube, add gradually 2 c.c. of the test-solution. A red color develops of an intensity in proportion to the amount of nitrites present. The appearance of the color may be hastened by heating.

If this test shows the presence of nitrites, test one-half of the remaining solution for ammonia with Nessler's solution.¹

The presence of ammonia is shown by the immediate development of a yellow color or precipitate on the addition of a few drops of the test-solution.

When these tests are positive our inquiry has been answered. When negative the nitrates may have remained unchanged or may have been reduced to free nitrogen. It is therefore necessary to determine whether the nitrates are still present or not, as follows :

(a) Invert the tube containing the culture and evaporate to dryness the small amount of culture remaining on the inside of the tube.

¹ See Appendix, page 174.

(*b*) Add, in order, phenol-sulphonic acid,¹ water, and sodium hydrate. A yellow color shows the presence of nitrates.

(*j*) **Acid or Alkali Production.**—For the determination of acid or alkali production, cultures are made on solid media to which have been added 1 per cent. of dextrose, saccharose, or lactose, and a sufficient quantity of litmus solution to produce a blue tinge. A pink coloration of the colony or a reddening of the surrounding medium indicates acid production.

(*k*) **Test for Indol Production.**—For the determination of indol production, incubate the species to be tested in the dextrose-free bouillon described on page 56. Add to the tube of culture 1 c.c. of a 0.01–0.02 per cent. solution of sodium or potassium nitrite. To each 10 c.c. of medium add one drop of concentrated sulphuric acid. A red coloration indicates indol. If no result is obtained at once, it is well to allow the tube to stand for one hour.

Pathogenesis.—In Chapter VIII. are described the various methods of determining the pathogenic properties of bacteria.

1. When a given form grows only at or below 18° to 20° C. inoculation should be made into the dorsal lymph-sac of a frog, using about 1 per cent. of the body-weight of a bouillon culture seven days old.

2. When a given species grows at 35° C. or upward, inoculation should be made into the peri-

¹ See Appendix, page 174.

toneal cavity of a white or ordinary house mouse, using 1 per cent. of the body-weight of a forty-eight hour bouillon culture. If the mouse is killed, it is well to try the culture on guinea-pigs or rabbits. A careful autopsy should be made in all cases, cultures taken from the organs, and all pathogenic changes noted.

II. DETERMINATION OF THE NAME OF A SPECIES.

When the points mentioned at the beginning of this chapter have been determined, the name of the species, if it has been described and named, can be found by referring to the bacterial analysis tables in Migula's *System der Bakterien*, Sternberg's *Manual of Bacteriology*, Flügge's *Die Mikroorganismen*, or Chester's "Studies in Systematic Bacteriology" in the ninth, tenth, and eleventh *Reports of the Delaware College Agricultural Experiment Station*, 1897, 1898, and 1899.

The following table, adapted from Migula, may assist in the determination of the name of a species :

BACTERIA.

I. Family. Coccaceæ.

I. Genus. Streptococcus.

I. Grow on gelatin.

I. Colonies white.

A. Do not liquefy gelatin.

(a) No growth on the surface in gelatin stab cultures.

S. pyogenes.

S. equi.

S. cystitidis.

S. urinæ.

S. acidi lactici.

(*b*) Growth on the surface and along the
puncture in gelatin stab cultures.

S. mastitidis.

S. mirabilis.

B. Liquefy gelatin.

S. septicus.

S. morbi brightii.

S. gracilis.

S. vermiformis.

S. albus.

2. Colonies colored.

S. cerasinus.

S. citreus.

II. Do not grow on gelatin.

S. giganteus.

2. Genus. *Micrococcus.*

I. Grow on gelatin.

1. White.

A. Do not liquefy gelatin.

M. candicans.

M. tardissimus.

M. tardus.

M. plumosus.

M. viticulosus.

M. stellatus.

M. tenuissimus.

M. albocereus (*cereus albus*).

M. ureæ.

M. coryzæ.

M. salivarius.

M. lacticus.

M. acidi lactici.

M. phosphoreus.

M. catarrhalis.

M. bovis.

M. similis.

B. Liquefy gelatin.

M. pyogenes.

M. ovis.

M. foetidus.

M. conoideus.

M. liquefaciens.

M. radiatus.

M. faviformis.

M. amplus.

M. dissimilis.

2. Yellow.

A. Do not liquefy gelatin.

M. aurantiacus.

M. tardigradus.

M. luteus.

M. cereus.

M. versicolor.

M. varians.

B. Liquefy gelatin.

M. aureus (*S. pyogenes aureus*).

M. beri-beri.

M. fuscus.

M. coronatus.

M. conjunctivitis.

M. tardus.

M. flavus.

- M. desidens.
- M. conglomeratus.
- M. citreus (S. pyogenes citreus).
- M. citrinus.
- M. corrugatus.
- M. mollis.

3. Red.

A. Do not liquefy gelatin.

- M. cinnabareus.
- M. carneus.

B. Liquefy gelatin.

- M. roseus.
- M. rosaceus.
- M. carnicolor.
- M. fragilis.

4. Blue and violet.

- M. violaceus.
- M. cyaneus.

II. Do not grow on gelatin.

- M. gonorrhœæ.
- M. intracellularis.
- M. subflavidus.
- M. rugatus.
- M. cuniculorum.
- M. progrediens.
- M. tenuis.
- M. nitrosus.
- M. foetidus.

3. Genus. Sarcina.

I. Grow on gelatin.

1. White.

- Sarcina alba.

2. Yellow.

A. Do not liquefy gelatin.

*Sarcina lutea.**Sarcina ventriculi.*

B. Liquefy gelatin.

*Sarcina liquefaciens.**Sarcina aurantiaca.*II. Family. *Bacteriaceæ.*I. Genus. *Bacterium.*

I. Form spores.

1. Spores polar.

*Bact. anthracis.**Bact. anthracoides.**Bact. subtile.*

2. Spores central.

*Bact. carotarum.**Bact. angulans.*

3. Position of spores undetermined.

A. Grow on gelatin at the room-temperature.

(a) Colonies white.

1. Do not liquefy gelatin.

*Bact. acidi lactici.**Bact. coprogenes.*

2. Liquefy gelatin.

(1) Center of colonies plainly floccose.

Bact. vermiculare.

(2) Colonies not floccose.

*Bact. tricomii.**Bact. nephritidis.**Bact. sempervivum.**Bact. lacteum.*

(b) Colonies colored.

Bact. brunneum.

B. Do not grow on gelatin at the room-temperature.

Bact. termophilum.

II. Spore formation not observed.

I. Grow well on gelatin.

A. Form no pigment.

(a) Do not liquefy gelatin.

1. Stained by Gram's method.

Bact. pneumoniae.

Bact. proteus.

Bact. rhusiopathiae.

Bact. murisepticum.

Bact. lacticum.

Bact. parvum.

2. Do not stain by Gram's method.

Bact. pneumonicum.

Bact. rhinoscleromatis.

Bact. faschingii.

Bact. endocarditidis.

Bact. cuniculicidi.

Bact. suicida.

Bact. palumbarium.

Bact. ribberti.

Bact. cuniculi.

Bact. pseudotuberculosis.

Bact. columbarum.

Bact. aërogenes.

Bact. aceti.

Bact. salivæ.

3. Gram's stain undetermined.

Bact. capsulatum.

Bact. keratomalaciæ.

Bact. felis.

Bact. pyogenes.

Bact. welchii (aërogenes capsu-
latus).

Bact. bienstockii.

Bact. laerii.

Bact. ubiquitum.

Bact. candicans.

Bact. phosphorescens.

Bact. giardi.

(b) Liquefy gelatin.

Bact. bovis.

Bact. vaginalis.

Bact. varicosum.

Bact. buccale.

B. Colonies yellow.

(a) Do not liquefy gelatin.

Bact. erythrogenes.

Bact. citreum.

Bact. aurescens.

(b) Liquefy gelatin.

Bact. arborescens.

Bact. aquatile.

Bact. chlorinum.

Bact. aureum.

C. Colonies red.

Bact. mycoides.

Bact. pyocinnabareum.

D. Colonies blue or violet.

Bact. cœruleum.

Bact. amethystinum.

2. Do not grow well on gelatin at room-temperature.

Bact. tuberculosis.

Bact. tuberculosis avium.

Bact. lepræ.

Bact. syphilidis.

Bact. smegmatis.

Bact. mallei.

Bact. diphtheriæ.

Bact. xerosis.

Bact. pseudodiphtheriticum.

Bact. vaginæ.

Bact. influenzæ.

Bact. pseudoinfluenzæ.

2. Genus. *Bacillus*.

- I. Form spores.

1. Spores polar.

B. ramosus.

2. Spores central or oblique.

B. subtilis.

B. megaterium.

3. Position of spores undetermined.

A. White or dirty white on gelatin.

(a) Do not liquefy gelatin.

B. spermophilinus.

B. polypiformis.

B. muscoides.

(b) Liquefy gelatin.

1. Stain by Gram's method.

B. mycoides.

B. gracilis.

B. granulosus.

B. tetani (anaërobic).

2. Gram's stain undetermined.

B. cereus.

B. lævis.

B. vermicularis.

B. thalossophilus.

B. intricatus.

B. limophilus.

B. circulans.

B. pseudanthracis.

B. globigii.

B. mesentericus.

B. vulgatus.

B. sporogenes.

B. liodermos.

B. albolactus.

B. butyricus.

B. chauvæi (anaërobic).

B. radiatus (anaërobic).

B. œdematis.

B. Form a black pigment on gelatin.

B. aterrimus.

B. niger.

II. Spore formation not observed.

1. Grow on gelatin. Not phosphorescent.

A. Colonies white.

(a) Do not liquefy gelatin.

1. Stain by Gram's method.

B. zenkeri.

B. muripestifer.

2. Do not stain by Gram's method.

B. typhosus.

B. coli.
B. pestis.
B. avium.
B. suipestifer.
B. zeæ.
B. glacialis.

3. Gram's stain undetermined.

B. murium.
B. solanacearum.
B. phaseoli.
B. amylovorus.
B. sorghi.
B. zopfii.

(b) Liquefy gelatin.

1. Stain by Gram's method.

B. dysenteriae.

2. Do not stain by Gram's method.

B. pseudotuberculosis.

B. ozænae.

B. vulgaris.

B. halophilus.

3. Gram's stain undetermined.

B. arthuri.

B. sulfureus.

B. liquidus.

B. diffusus.

B. nubilus.

B. reticularis.

B. diaphanus.

B. mirabilis.

B. gasoformans.

B. delicatulus.

- B. cloacæ.
- B. hyalinus.
- B. superficialis.
- B. Colonies yellow.
 - B. arborescens.
- C. Colonies red.
 - B. prodigiosus.
 - B. indicus.
 - B. plymouthensis.
 - B. rubescens.
- 2. Grow on gelatin. Phosphorescent.
 - B. phosphorescens.
 - B. fischeri.
 - B. phosphoricus.
- 3. Do not grow on gelatin.
 - B. equi.
- 3. Genus. *Pseudomonas*.
 - I. Grow on gelatin.
 - 1. Colonies white. Form no pigment.
 - P. litoralis.
 - 2. Form fluorescent pigment.
 - A. Do not liquefy gelatin.
 - P. alba.
 - P. tenuis.
 - P. eisenbergii.
 - P. stewarti.
 - B. Liquefy gelatin.
 - P. æruginosa (B. pyocyaneus).
 - P. fluorescens.
 - P. minutissima.
 - 3. Colonies blue or violet.
 - P. ianthina.

- P. pseudianthina.
- P. laurentia.
- 4. Phosphorescent species.
 - P. javanica.
- II. Do not grow on gelatin.
 - P. europæa.
 - P. javaniensis.
- III. Family Spirillaceæ.
 - 1. Genus. Spirosoma.
 - Spirosoma nasale.
 - Spirosoma linguale.
 - Spirosoma aureum.
 - Spirosoma flavum.
 - Spirosoma flavescens.
 - Spirosoma attenuatum.
 - Spirosoma gregarium.
 - 2. Genus. Microspira.
 - I. Not phosphorescent.
 - 1. Do not liquefy gelatin.
 - Microspira canalis.
 - Microspira saprophiles.
 - Microspira tonsillaris.
 - 2. Liquefy gelatin.
 - Microspira comma.
 - Microspira metschnikovi.
 - Microspira finkleri.
 - Microspira sputigena.
 - Microspira marina.
 - II. Phosphorescent.
 - Microspira dunbari.
 - Microspira coronata.
 - Microspira annularis.

Microspira glutinosa.
Microspira delgadensis.
Microspira tuberosa.
Microspira degenerans.
Microspira luminosa.
Microspira caraibica.
Microspira papillaris.

III. CLASSIFICATION OF BACTERIA BY GROUPS.

Systematic bacteriology is at present in a state of chaos. Many times the most that can be done in attempting to classify a new species, is to refer it to some group, the members of which have certain characters in common, and are probably the descendants of one ancestral type. Chester has proposed¹ a synopsis of several groups of bacteria which, slightly modified, is given below.

BACTERIUM.

I. Spore-formers.

1. No growth at room-temperature or below 22°-25° C.

THERMOPHILIC GROUP.

Bact. termophilum type.

2. Grow at room-temperature.

A. Do not liquefy gelatin.

BACT. FÆCALIS GROUP.

Bact. subtile type.

B. Liquefy gelatin.

¹ *Eleventh Annual Report of the Delaware College Agricultural Experiment Station for 1898-99.*

ANTHRAX GROUP.

Bact. anthracis type.

II. Spore formation not observed. Aërobic and facultative anaërobic.

1. Do not liquefy gelatin.

A. Do not stain by Gram's method.

(a) Obligate aërobic.

ACETIC FERMENT GROUP.

Bact. aceti type.

(b) Aërobic and facultative anaërobic.

1. Gas generated in glucose bouillon.

a. Gas generated in lactose bouillon.

BACT. AËROGENES GROUP.

Bact. aërogenes type.

b. Little or no gas generated in lactose bouillon.

FRIEDLANDER GROUP.

Bact. pneumonicum type.

2. No gas generated in glucose bouillon.

a. Coagulate milk.

FOWL-CHOLERA GROUP.

Bact. cuniculcida type.

b. Do not coagulate milk.

SWINE-PLAGUE GROUP.

Bact. suicida type.

B. Stain by Gram's method.

Gas generated in glucose bouillon.

LACTIC FERMENT GROUP.

Bact. lacticum type.

2. Liquefy gelatin.

GLANDERS GROUP.

Bact. mallei type.

3. Do not grow well on gelatin at room-temperature.

A. Stain with basic aniline dyes, but are easily decolorized by mineral acids when stained with carbol-fuchsin.

- (a) Grow well in bouillon at body-temperature and stain by Gram's method.

DIPHTHERIA GROUP.

Bact. diphtheriæ type.

- (b) Do not grow in bouillon or on ordinary media.

1. Rods slender.

a. Stain by Gram's method.

LEPROSY GROUP.

Bact. lepræ type.

- b. Do not stain by Gram's method.

INFLUENZA GROUP.

Bact. influenzæ type.

2. Rods variable.

ROOT-TUBERCLE GROUP.

- B. Do not stain with aqueous solutions of basic anilins and are not easily decolorized by acids.

TUBERCLE GROUP.

Bact. tuberculosis type.

BACILLUS.

- I. Spore formers.

1. Aërobic and facultative anaërobic. Rods not swollen at sporulation.

A. Liquefy gelatin slowly.

UROBACILLUS GROUP OF MIQUEL.

B. Liquefy gelatin quickly.

(a) Potato cultures rugose.

POTATO-BACILLUS GROUP.

B. mesentericus type.

(b) Potato cultures smooth.

B. SUBTILIS GROUP.

B. subtilis type.

2. Obligate anaërobic.

A. Rods not swollen at sporulation.

MALIGNANT EDEMA GROUP.

B. chauvæi type.

B. Rods clavate at sporulation.

TETANUS GROUP.

B. tetani type.

II. Spore formation not observed.

1. Aërobic and facultative anaërobic.

A. Gelatin colonies roundish, not distinctly ameboid.

(a) Do not liquefy gelatin.

1. Do not stain by Gram's method.

a. Generate gas in glucose bouillon.

(1) Coagulate milk.

COLON GROUP.

B. coli type.

(2) Do not coagulate milk.

HOG-CHOLERA GROUP.

B. suipestifer type.

- b.* Do not generate gas in glucose bouillon.

TYPHOID GROUP.

B. typhosus type.

- 2. Stain by Gram's method.

B. MURIPESTIFER GROUP.

B. muripestifer type.

- (*b*) Liquefy gelatin and generate gas in glucose bouillon.

B. CLOACÆ GROUP.

B. cloacæ type.

- B.* Gelatin colonies ameboid or irregular.

- (*a*) Do not liquefy gelatin.

B. ZOPFI GROUP.

B. zopfi type.

- (*b*) Liquefy gelatin.

PROTEUS GROUP.

B. vulgaris type.

MICROSPIRA.

- I. Not phosphorescent.

- 1. Do not liquefy gelatin, or only slightly.

MSP. SAPROPHILES GROUP.

Msp. saprophiles type.

- 2. Liquefy gelatin.

- A. Produce indol.

- (*a*) Very pathogenic to pigeons.

MSP. METSCHNIKOV GROUP.

Msp. metschnikovi type.

(b) Not distinctly pathogenic to pigeons.

CHOLERA GROUP.

Msp. comma type.

B. Do not produce indol, or very little, at least after twenty-four hours.

CHOLERA-NOSTRAS GROUP.

Msp. finkleri type.

IV. CLASSIFICATION OF WATER BACTERIA BY GROUPS.

Fuller and Johnson have applied the group method of classification to the bacteria of water as follows :¹

WATER BACTERIA.

I. Fluorescent.	Group I.
II. Non-fluorescent.	
1. Chromogenic.	
A. Red.	Group II.
B. Orange.	Group III.
C. Yellow.	Group IV.
D. Violet.	Group V.
2. Non-chromogenic.	
A. Gelatin liquefied.	
(a) Characteristic colonies on gelatin plates.	

¹ *Jour. of Exp. Med.*, vol. iv., Nos. 5-6, p. 609. Conn has adopted a somewhat similar classification into groups for the dairy bacteria. See *Report of the Storrs (Connecticut) Agricultural Experiment Station for 1899*.

1. Proteus forms. Group VI.
2. Subtilis forms. Group VII.
- (b) Non-characteristic colonies on gelatin plates.
 1. Fermentation of carbohydrates.
 - a. Gas production. Group VIII.
 - b. Nogas production. Group IX.
 2. Non-fermentation of carbohydrates. Group X.
- B. Gelatin not liquefied.
 - (a) Fermentation of carbohydrates.
 1. Gas production. Group XI.
 2. No gas production. Group XII.
 - (b) Non-fermentation of carbohydrates. Group XIII.

CHAPTER IX.

BACTERIAL ANALYSIS OF WATER, MILK, AIR, AND SOIL.

WATER ANALYSIS.

THE biologic examination of water is for the purpose of determining the number and kinds of organisms present. It serves to supplement chemical analysis, and both are necessary to arrive at a sound conclusion as to potability.

I. Quantitative Analysis of Water.—The number of bacteria present in water varies within wide limits without affecting the value of the water; consequently no fixed standard can be of much value in determining the quality. The proper study of a water-supply should include the determination of its normal mean number of bacteria at every season of the year. Any variation from the mean can then readily be determined, and its cause investigated. If simultaneous analyses of waters from various sources are made, the preferable water for drinking-purposes can easily be selected. Beyond this the value of the water can be determined by quantitative analysis alone only within wide limits. Water containing less than 100 bacteria per cubic centimeter is presumably from a deep source and uncontaminated by surface drainage. It can usually be recommended for drinking-

purposes. Water containing more than 500 per cubic centimeter should be looked upon with suspicion.

2. Qualitative Analysis of Water.—The qualitative examination of water requires not only the isolation of the several species present, but also their cultivation and the determination of their pathogenic or non-pathogenic properties. Such an examination takes a long time, and under most favorable circumstances it is very difficult to recognize the presence of pathogenic forms.

The principal value of the qualitative analysis of water is in the detection of contamination by sewage. Sewage is always liable to contain the evacuations of patients sick with typhoid fever or other transmissible diseases. The germs of typhoid fever are not easily identified, but there are certain bacteria common in human and other animal evacuations and in sewage (*B. coli*, *B. vulgaris*, *B. cloacæ*, *B. sporogenes*, *Bact. aërogenes*) whose presence is easily detected. Consequently the presence of such forms, though harmless in themselves, always indicates contamination.

3. Laboratory Work in Water Analysis.—All samples should be collected in sterile flasks, and cultures should be made immediately to secure accurate results. If transportation is necessary, the samples should be packed in ice. Tap water should be allowed to run a few minutes before the sample is taken; if spring or well water is to be examined, the sample should be collected from about a foot below the surface.

(a) Transfer 1 c.c. of the water to be examined, by means of a sterile graduated pipet, to each of three tubes of liquefied gelatin.¹ Use a sterile pipet for each transfer.

(b) Shake the tubes, flame the lips, and pour into sterile Petri dishes. Place these in the dark at 20° C.

(c) Examine the plates from day to day, and count the colonies that appear.

FIG. 50.—Simple microscope for counting colonies.

It is best to count all the colonies if possible ; but when they are very numerous, some one of the various methods devised for counting colonies must be employed.

(1) Wolfhügel's counting-plate consists of a glass plate on which are ruled square centimeters. The Petri dish is

¹ If the water is suspected of containing large numbers of bacteria, smaller quantities than 1 c.c. should be added to each tube, or, better, dilute the water by the addition of a known quantity of sterile water. Many workers prefer to mix the water and the gelatin in the Petri dish instead of in the tube.

placed thereon and the number of colonies in several of the square divisions counted, the average taken, and the

FIG. 51.—Wolfhügel's apparatus for counting colonies of bacteria upon plates.

number in the whole dish estimated. A lens is used in counting the colonies.

FIG. 52.—Jeffer's plate for counting colonies in circular dishes. The area of each division is 1 square centimeter.

(2) Colonies in circular dishes may be counted by means of Jeffer's plate. In this each circle is marked with its area in square centimeters, and each division equals 1 square centimeter.

(3) The colonies appearing under the microscope in the field of a low-power objective may be counted, in several parts of the dish, the average taken, and the number in the whole dish estimated by the equation :

$$\frac{\text{Number of colonies in the field}}{\text{Whole number of colonies}} = \frac{(\frac{1}{2} \text{ diam. of field})^2}{(\frac{1}{2} \text{ diam. of dish})^2}$$

(d) If a qualitative analysis is required, isolate and cultivate the different kinds of colonies.¹

4. Test for the Presence of *Bacillus coli*.—

(a) Prepare 10 fermentation-tubes of sterile bouillon containing 1 per cent. glucose.

(b) To each tube add 1 c.c. of the water to be tested.

(c) Place the tubes in the incubator at 37.5° C. for three days.

(d) Note the amount of gas which forms on each of the three days.

If gas-forming bacteria are present, gas will collect in the closed tube. The number of tubes showing the presence of gas gives a rough idea of the number of gas-producing bacilli present.

Bacillus coli, if present, will fill the closed tube by the second day. Too little or too much gas does not point to the presence of *Bacillus coli*. *Bacillus coli* forms most of its gas during the first twenty-four hours. The liquid in the bulb must

¹ See p. 112 for Fuller and Johnson's groups of water bacteria.

be distinctly acid to indicate the presence of *Bacillus coli*.

5. Isolation of *Bacillus coli*.—First Method.—

(a) Add 50 c.c. of the water to be tested to 50 c.c. of sterile bouillon in a sterile flask.

(b) Place in the incubator at 37.5° C. for two days.

The high temperature will destroy the common water bacteria, but will encourage the growth of the coli group.

(c) Test part of this culture for indol. Its presence indicates the presence of *Bacillus coli*.

(d) Make plates from this culture.

(e) If any non-liquefying colonies are whitish with irregular leafy outlines and show lines more or less radial, they are probably colonies of *Bacillus coli*, and must be carefully studied in cultures.¹

Second Method.—(a) Add 70 c.c. of the water to be tested to 30 c.c. of sterile bouillon.

(b) To the mixture add 1 c.c. of 5 per cent. carbolic acid.

(c) Incubate at 37.5° C. for twenty-four hours. Carbolic acid restrains the growth of the ordinary water bacteria, while the coli group and other intestinal forms grow unhindered.

(d) From the growth that results inoculate fermentation-tubes containing 1 per cent. glucose bouillon. Note the amount of gas that forms as before.

(e) Make plates from the growth in the tubes,

¹ See page 60.

and study the colonies that resemble those of *Bacillus coli*.

6. Milk Analysis.—The analysis of milk is conducted in the same manner as is that of water, but on account of the great number of bacteria in

FIG. 53.—Hesse's apparatus for collecting bacteria from the air.

milk the samples must be diluted with sterile water.

Bacillus typhosus and *Bacillus coli* are detected in milk by the same methods as in water analysis. Tuberculosis bacilli may be detected in milk by the method described in the next chapter.¹

¹ See page 157.

For the classification of bacteria in milk, see H. W. Conn, *Report of the Storrs (Connecticut) Agricultural Experiment Station for 1899*.

FIG. 54.—Petri's sand-filter for air examination.

FIG. 55.—Sedgwick's expanded tube for air examination.

7. Bacteria in the Air.—(a) Prepare 3 gelatin plates and expose them to the air for four or five

minutes in different places. (In recitation-rooms before and after class, out of doors, etc.)

(*b*) Cover and allow to grow.

(*c*) Examine from day to day, and make cultures from the different colonies.

This is a rough method of determining the relative number of bacteria in the air. For more exact results recourse must be had to special apparatus for aspirating through bouillon or through sugar, as described in the text-books.

8. Bacteria in the Soil.—Numerous species of

FIG. 56.—Fränkel's instrument for obtaining earth from various depths for bacteriologic examination.

bacteria occur in the soil; some are of special interest on account of their pathogenic properties. Many are anaërobic, and this fact must be kept in mind while studying them.

To determine the number of bacteria in a sample of soil :

(*a*) Collect the soil without contamination from bacteria from other sources.

(*b*) Introduce a measured quantity into a tube of liquefied gelatin. Crush with a platinum needle, and mix thoroughly with the medium.

(*c*) Make plates, count, isolate, and study in the usual way.

Another method, and one which does away with the presence of particles of soil in the medium, but which perhaps does not give such accurate results, is to mix the sample thoroughly with sterile water and then make plates from the water.

CHAPTER X.

PATHOGENIC BACTERIA.

THE methods for the study of pathogenic bacteria are exactly the same as those already described. In certain cases only are special culture-media necessary for their growth. Most pathogenic forms grow better in the incubator at body-temperature, 37.5° C. In all cases animal inoculations are necessary for the determination of pathogenicity.

FIG. 57.—*Micrococcus aureus*, from an agar-agar culture (Günther).

Great care must be used in handling pathogenic cultures in order to avoid accidents. Have at hand a solution of corrosive sublimate (1 : 1000) or carbolic acid (1 : 20), with which to flood any material that

may by accident be spilled on floor or table. Carefully sterilize everything that the pathogenic material may have contaminated. Thoroughly disinfect the hands, instruments, and table with the corrosive sublimate solution after completing the work.

FIG. 58.—*Streptococcus pyogenes* (from a bouillon culture).

I. PYOGENIC ORGANISMS.

(a) Study, according to the schedule in Chapter VIII., the morphology and biology of :

- Micrococcus aureus* (*Staphylococcus pyogenes aureus*).
- Micrococcus citreus* (*Staphylococcus pyogenes citreus*).
- Micrococcus pyogenes* (*Staphylococcus pyogenes albus*).
- Streptococcus pyogenes*.
- Sarcina tetragena* (*Micrococcus tetragenus*).

(b) Select and weigh a well-grown rabbit. Inoculate into the ear vein by means of a hypodermic syringe¹ 1 c.c. of a twenty-four or forty-eight hour bouillon culture of one of the above species, or of pus taken directly from an abscess.

¹ Keep the syringe for some time before the operation in a 2 per cent. solution of carbolic acid. Wash it out five or six times with sterile water or bouillon before the inoculation; or the syringe and needle may be boiled for five minutes before using.

FIG. 59.—Koch's syringe.

(c) Record the daily weight of the animal until death.

(d) Perform an autopsy on the dead rabbit, note

FIG. 60.—Method of making an intravenous injection into a rabbit. Observe that the needle enters the posterior vein from the hairy surface (McFarland).

the various pathologic changes, and make cultures and cover-glass smears from all the organs and from the blood.¹

¹Sear the surface of the body-wall and of each viscus with a red-hot spatula or old scalpel before cutting into

(e) Preserve some of tissues in absolute alcohol or in Zenker's fluid.¹

(f) Stain the smear preparations and examine for the inoculated organism.

(g) Make pure cultures from whatever growth is

FIG. 61.—*Streptococcus pyogenes*; cover-glass preparation from the pus of an abscess; $\times 1000$ (Fränkel and Pfeiffer).

obtained in the cultures from the blood and organs, and try to recover the original organism.

them; sterilize the blades of the knives, scissors, and forceps used in making the incisions by dipping them in methyl alcohol and passing them quickly near enough to a Bunsen flame to ignite the alcohol. Smears and cultures from the blood are best made from the blood in the heart. For the method of preparation of blood-smears, see page 59.

¹ See Appendix, page 173.

FIG. 62.—*Streptococcus pyogenes*, seen in a section through human skin ; $\times 500$ (Fränkel and Pfeiffer).

FIG. 63.—*Sarcina tetragena* in pus from a white mouse ; $\times 615$ (Heim).

(h) Section the preserved tissue and stain for bacteria with carbol-thionin-blue, Kuhne's methylene-blue, or by Gram's method.

FIG. 64.—Mouse-holder, with mouse in position for inoculation.

Other animals—guinea-pigs, rats, or mice—may be used for inoculations. Inoculations may be subcutaneous, peritoneal, or intravenous, according to the organisms used or the nature of the experiment.

II. GONOCOCCUS.

1. Examine cultures of *Micrococcus gonorrhææ*, and stain the organism with Löffler's blue and by Gram's method.

2. Examine gonorrheal pus as follows :

- (a) Prepare films on cover-glasses.
- (b) Pass three times through flame.
- (c) Stain with Löffler's methylene-blue or with any aqueous anilin stain one minute.
- (d) Wash, dry, and mount.
- (e) Examine with the oil-immersion lens.

Gonococci are of medium size, composed usually of two hemispheres separated by a narrow unstained interval. Occasionally two pairs of cocci form a "tetrad." The cocci are usually within the leukocytes.

(f) Stain another film by Gram's method. The gonococci are decolorized.

III. ANTHRAX.

1. Study the morphology and biology of cultures of *Bacterium anthracis*.
2. Inoculate a guinea-pig as follows :

FIG. 65.—Gonococcus in urethral pus; $\times 1000$ (Fränkel and Pfeiffer).

(a) Remove the hair from a small area on the abdomen.

(b) With a snip of a pair of sterile scissors make a little subcutaneous pocket.

(c) Introduce into this pocket spores and bacteria from a pure culture by means of a platinum loop.

(a) At the autopsy prepare cultures, smears, and sections from the organs.

FIG. 66.—*Bacterium anthracis*; colony three days old upon a gelatin plate; adhesive preparation; $\times 1000$ (Fränkel and Pfeiffer).

(e) Make cover-glass preparations from the blood as follows:

1. Place a small drop of blood between two absolutely clean cover-glasses, draw them apart and allow the smears to dry.

2. Fix in equal parts of ether and alcohol for thirty minutes, or in absolute alcohol five minutes,

or in vapor of formaldehyd two and a half minutes; or heat in the thermostat at 110° – 120° C. for twelve hours; or heat on a brass plate for one hour at the point where water boils.

FIG. 67.—*Bacterium anthracis*; cover-glass preparation from the spleen of a mouse (Mallory and Wright).

3. Stain in eosin ($\frac{1}{2}$ per cent. in 60 per cent. alcohol) from one to five minutes.
4. Wash in water and dry.
5. Contrast-stain in aqueous methylene-blue from one-half to one minute.
6. Wash, dry, and mount.

IV. GLANDERS.

1. Study the morphology and biology of the glanders bacterium.
2. Inoculate a male guinea-pig intraperitoneally with 1 c.c. of a bouillon culture.
3. Note in two or three days the great swelling

FIG. 68.—Bacterium anthracis, stained to show spores ;
× 1000 (Fränkel and Pfeiffer).

and redness of the testicles, caused by a semipurulent affection of the tunica vaginalis. This is a diagnostic test for the glanders bacterium.

4. At the autopsy prepare smears, cultures, and sections from all the organs and from the peritoneal and scrotal nodules.

5. Stain sections as follows :

- (a) Carbol-thionin-blue for ten to fifteen minutes.
- (b) Wash thoroughly in water.
- (c) Dehydrate in anilin oil.
- (d) Treat with equal parts of anilin oil and xylol.
- (e) Pass through pure xylol to balsam.

FIG. 69.—*Bacterium mallei*, from a culture upon glycerin agar ; $\times 1000$ (Fränkel and Pfeiffer).

6. For the diagnosis of a suspected case of glanders proceed as follows :

(a) Rub a large swab made of absorbent cotton in the discharge from the nose or in the suspected ulcer.

(b) Transfer the swab to 5 c.c. of sterile water and shake thoroughly.

(c) Inoculate the resulting suspension intraperitoneally into a well-grown male guinea-pig.

(*d*) In two to seven days scrotal inflammation will develop if the glanders bacterium was present. If the glanders bacterium was not present, after a few hours or days of depression the guinea-pig recovers completely. It may happen that some acute septic organisms were present in the material injected. In such cases the guinea-pig usually dies within twenty-four hours, and the test is evidently of no value and must be repeated.

(*e*) If the scrotal lesions appear, perform an autopsy. Look particularly for nodular deposits in the peritoneum and visceral layer of the tunica vaginalis, infiltration of the scrotal tissue, and edema extending into the groin and suprapubic region.

(*f*) Transfer aseptically a portion of a nodule to potato, and place at 37.5° C. In twenty-four to forty-eight hours small, smooth, glistening, amber-colored colonies of the glanders bacterium should develop. The bacterium is a short, thick rod, with rounded ends, usually slightly curved or bent, sometimes elongated into threads. It stains faintly in Löffler's methylene-blue.

(*g*) A positive diagnosis can usually be made from the gross lesions, but the isolation of the organism makes the diagnosis certain.

V. DIPHTHERIA.

1. Make swabbings from the throats of healthy individuals and from several diphtheria patients in hospital. Depress the tongue and rub a sterile

FIG. 70.

FIG. 70.—Providence Health Department outfit for diphtheria diagnosis. A pasteboard box containing a swab-tube and a serum-tube, both with etched surface on which to write the name and address of patients, etc.

FIG. 71.

FIG. 71.—*Bacterium diphtheriæ*; agar culture (photograph by Dr. Henry Koplik).

swab made of non-absorbent cotton over the back of the throat, tonsils, diphtheritic membrane, etc.

2. Rub the swab over the surface of a Löffler blood-serum tube.

3. Place the tube in an incubator at 37.5° C. for sixteen hours or longer.
4. Examine the growth for the small grayish, slightly elevated diphtheria colonies.
5. Prepare films from a suspected colony.
6. Pass three times through the flame and stain in Löffler's methylene-blue for one minute.

FIG. 72.—*Bacterium diphtheriæ*, from culture upon blood-serum ; $\times 1000$ (Fränkel and Pfeiffer).

7. Wash, dry, mount, and examine with the $\frac{1}{12}$ inch oil-immersion lens. The characteristic diphtheria bacteria can easily be detected.
8. Isolate the diphtheria organisms in pure culture by inoculating tubes from a single colony which on examination proves to be diphtheria. If a single colony cannot be found, touch the needle

once to the growth on the serum-tube and make a series of strokes on 3 or 4 tubes. When the colonies develop, those in the last tube will be sufficiently distinct so that pure cultures may be made from them. Another method is to inoculate a bouillon-tube or a tube of sterile water or normal salt solution, and immediately from this make stroke cult-

FIG. 73.—*Bacterium diphtheriæ*, colony twenty-four hours old upon agar ; $\times 100$ (Fränkel and Pfeiffer).

ures on serum-tubes. The growth on these will probably be in individual colonies. Either of these methods obviates the necessity of making plates.

9. Study the morphology and biology of the diphtheria bacterium obtained above.

10. The following stains are diagnostic for the diphtheria bacterium :

Hunt's Stain.

- (a) Prepare films as usual.
- (b) Stain in aqueous methylene-blue for one minute.
- (c) Wash in water and dry.
- (d) Treat with a 10 per cent. solution of tannic acid for one minute.
- (e) Wash in water and dry.
- (f) Stain in aqueous methyl-orange for one minute.
- (g) Wash, dry, and mount.

Neisser's Stain.

- (a) Prepare solution A as follows :

Methylene-blue,	1 gm. ;
Alcohol (95 per cent.),	20 c.c.

Dissolve and add

Acetic acid,	50 c.c. ;
Water,	950 c.c.

- (b) Prepare solution B as follows:

Bismarck-brown,	2 gm. ;
Boiling water,	1000 c.c.

- (c) Treat films with solution A for one to three seconds.
 - (d) Wash in water.
 - (e) Treat with solution B for three to five seconds.
 - (f) Wash, dry, and mount.
- II. Test the virulence of the diphtheria organism isolated as follows :

(a) Prepare a twenty-four to forty-eight hour bouillon culture.

(b) Sterilize a hypodermic syringe and needle by soaking in 2 per cent. carbolic acid and washing out in sterile water or bouillon, or by boiling for five minutes.

(c) Select and weigh a full-grown guinea-pig.

(d) While the pig is held on its back on the table

	60	59	58	57	56	55	54	53	52	51	
	41	42	43	44	45	46	47	48	49	50	
	30	39	38	37	36	35	34	33	32	31	
	21	22	23	24	25	26	27	28	29	30	
	20	19	18	17	16	15	14	13	12	11	
	1	2	3	4	5	6	7	8	9	10	

FIG. 74.—Slide, 7 x 2½ inches, for the routine examination of diphtheria cultures. Each square can be placed under the lens of the microscope without disturbing the equilibrium of the slide.

by an assistant, the hair is removed from a small area on the ventral abdominal wall.

(e) With the thumb and forefinger of the left hand pinch up a fold of the skin, and with the right hand insert the hypodermic needle between the skin and the muscular body-wall.

(f) Inoculate an amount of the culture equal to 1 per cent. of the weight of the pig.

(g) Record the daily weight of the pig until death.

(h) Perform an autopsy on the dead pig ; note the pathologic changes, and prepare cultures, smears, and sections from the organs and from the point of inoculation.

VI. INFLUENZA.

1. Prepare blood-agar tubes by smearing the surface of a ordinary agar-tube with a drop of blood

FIG. 75.—*Bacterium influenzae*, from a gelatin culture ;
× 1000 (Itzerott and Niemann).

obtained aseptically from man, rabbit, guinea-pig, pigeon, or frog.

2. Break up a distinctly purulent portion of influenza sputum in 1 or 2 c.c. of bouillon, and spread a loopful of the suspension over the surface of the blood-agar tube.

3. Place in the incubator and examine at the end of from eighteen to twenty-four hours.

4. The influenza colonies appear as minute colorless, glassy, transparent points, resembling drops of dew. They are barely visible to the unpractised eye, and require a low magnifying power to be seen clearly.

FIG. 76.—*Bacterium influenzæ*: colonies on blood-agar; low magnifying power (Pfeiffer).

5. Study the morphology and biology of the organisms in one of these colonies. They should not grow on ordinary media, and should have the morphology of the influenza bacteria.

6. Prepare smears from one of the purulent

masses in the sputum. Stain in very dilute carbol-fuchsin for five to ten minutes, or in Löffler's methylene-blue heated to the steaming-point. The influenza bacteria are very small, short, with

FIG. 77.—*Bacterium influenzæ* : cover-glass preparation of sputum from a case of influenza, showing the bacteria within the leucocytes ; highly magnified (Pfeiffer).

round ends, are often present in large numbers, and are frequently within the pus cells. They may occur in pairs, and then resemble cocci. The ends are usually more deeply stained than the central portions.

VII. TYPHOID AND COLON BACILLI.

FIG. 78.—*Bacillus typhosus*, from a twenty-four hour agar culture; $\times 650$ (Heim).

FIG. 79.—*Bacillus coli*, from an agar culture; $\times 1000$ (Itzerott and Niemann).

1. Study the morphology and biology of cultures

of *Bacillus typhosus* and *Bacillus coli*. Some of the differences between them are indicated below :

B. TYPHOSUS.	B. COLI.
Rods usually slender.	Rod inclined to be a little thicker.
Flagella more numerous, longer, more wavy (10-20).	Flagella fewer and shorter (8-10).
In artificial media growth generally slower and not so vigorous.	Growth faster and more vigorous.
Growth on fresh acid potato a nearly transparent film.	Growth on potato a brownish pellicle.
Very slight acid production in ordinary media, followed sometimes by a production of alkali.	Well-marked acid production.
Litmus milk—no change.	Litmus milk—pink color.
Milk not coagulated.	Milk coagulated.
Fermentation of lactose very slight if any.	Fermentation of lactose pronounced.
Litmus lactose agar—no change in color.	Litmus lactose agar—production of red color.
Glucose media—no gas formation.	Abundant gas formation.
No production of indol in ordinary bouillon.	Well-marked indol production in ordinary bouillon.
Does not grow in Maassen's asparagin-glycerin solution.	Grows in Maassen's solution.
Agglutination-test positive.	Agglutination-test usually negative.

2. Study sections of spleen, intestinal lesions, mesenteric lymph-glands, etc., from a human typhoid autopsy. Stain with carbol-thionin-blue or carbol-fuchsin.

3. **Widal Reaction in Typhoid Fever.**— If the blood-serum of a person suffering with typhoid fever or of one who has recently recovered from it

be added to a bouillon culture of actively motile typhoid bacilli, the bacilli lose their motility and

FIG. 80.—*Bacillus typhosus*: superficial colony two days old on a gelatin plate; $\times 20$ (Heim).

FIG. 81.—*Bacillus coli*: superficial colony two days old, on a gelatin plate; $\times 21$ (Heim).

soon aggregate in clumps. Dilutions of serum 1 : 10 and 1 : 30 with a half-hour time-limit are

those most commonly employed. In doubtful cases a dilution of 1 : 100 with a one-hour time-limit is recommended.

(a) Place 9 drops of a twenty-four-hour bouillon culture of actively motile typhoid bacilli on separate

FIG. 82.—*Bacillus typhosus*, from an agar culture six hours old, showing the flagella stained by Löffler's method ; $\times 1000$ (Fränkel and Pfeiffer).

spots on a clean cover-glass. Add 1 drop of serum from the blood of the suspected typhoid case,¹ mix

¹ The blood may be collected in a capillary tube and after coagulation the serum removed ; or dried blood may be moistened with water and the resulting solution used as serum. The latter method, however, does not permit accurate dilution. Chester and Robin have recently devised a pipet for delivering a measured drop of blood, so that dilu-

all together, and mount as a hanging-drop preparation.¹

(b) Examine with the $\frac{1}{6}$ inch or with the oil-

FIG. 83.—The Chester and Robin pipet for delivering uniform drops of blood for the Widal test. A glass and rubber pipet, the bulb of which is enclosed between two strips of metal held in place by a Hoffman clamp.

immersion lens. If the case is one of typhoid, after some time, varying between a few seconds and a

tions may be made with a fair degree of accuracy from dried blood. It consists of an ordinary medicine-dropper of a given size, the bulb of which is enclosed on either side by two narrow strips of metal (Fig. 83, *c, c.*), and both placed in a medium-sized Hoffman clamp. The inward movement of the clamp by means of the screw *a* compresses the bulb, while a slight turn in the opposite direction dilates it a little and permits a small drop of blood to enter. In expelling the blood the dropper is held vertically over a strip of thick filter-paper, and the clamp is slowly compressed until a single drop falls of its own weight. This drop is then dried, and when the test is to be applied the blood-spot is cut from the paper and drops of the diluting fluid are added from an exactly similar pipet until the required dilution is reached.

¹ It is convenient to use a slide on which two glass rings have been cemented, so that one may be used as a control, containing the culture of bacilli alone.

half-hour, the bacilli will be seen to become less motile, and finally to cease all movement and

FIG. 84.—Slide with two cells for observing Widal reaction with a control.

appear in clumps here and there throughout the field.

FIG. 85.—Widal reaction: *a*, bouillon culture of *Bacillus typhosus*; *b*, the same after the addition of typhoid serum.

This reaction can be reversed and made to serve as a means of identifying the typhoid bacillus if the serum of a person known to have typhoid fever is at hand.

VIII. PNEUMONIA.

BACTERIUM PNEUMONIÆ (FRANKEL'S PNEUMOCOCCUS)
AND BACTERIUM PNEUMONICUM (FRIEDLANDER'S
PNEUMOBACILLUS).

FIG. 86.—*Bacterium pneumoniae*, from the heart's blood of a rabbit; $\times 1000$ (Fränkel and Pfeiffer).

1. Study the morphology and biology of cultures of *B. pneumoniae* and *B. pneumonicum*.
2. Make cover-glass preparations from pneumonia sputum. Stain in Löffler's methylene-blue or carbol-fuchsin, diluted one-half.
3. Prepare sections of lung-tissue from cases of

lobar pneumonia, general infection, etc. Stain in carbol-thionin-blue and by Gram's method.

4. Inoculate a guinea-pig or rabbit subcutaneously or intravenously with virulent cultures or with

FIG. 87.—*Bacterium pneumonicum*, from the expectoration of a pneumonia patient ; $\times 1000$ (Fränkel and Pfeiffer).

pneumonia sputum ; also with sputum from healthy persons.

5. At the autopsy note the pathologic changes, prepare smears from the blood and organs, make cultures, and prepare sections.

IX. TUBERCULOSIS.

I. Examination of Cultures.

Study the morphology and biology of *Bacterium tuberculosis*.

(1) For cultures use blood-serum, agar, and bouillon to which from 4 to 8 per cent. of glycerin has been added.

(2) Stain films, prepared from cultures, with ordinary stains¹ and by the Ziehl-Neelson method as follows :

(a) Prepare films as usual.

(b) Stain in Ziehl's carbol-fuchsin, steaming but not boiling, five minutes ; cold, twenty minutes.

(c) Wash in 20 per cent. sulphuric acid for three to five seconds.

(d) Wash in 60 per cent. alcohol until no red color is left.

(e) Wash in water.

(f) Stain in aqueous solution of methylene-blue for one minute.

(g) Wash in water, dry, and mount.

The bacteria of leprosy, syphilis, and smegma stain by this method. *Bacterium lepræ* stains much more quickly than *B. tuberculosis*, *B. syphilidis* is decolorized more quickly, especially in sulphuric acid, and *B. smegmatis* is decolorized by the alcohol.

2. Examination of Tuberculous Sputum.

(1) Ziehl-Neelson Method.

(a) Place the sputum in a shallow glass dish on a black surface.

(b) Select several of the characteristic yellowish

¹ The tubercle bacillus takes the ordinary stains very slowly and faintly.

particles, place between two cover-glasses, and spread evenly.

(*c*) Draw the covers apart and allow the films to dry.

(*d*) Pass three times through flame.

FIG. 88.—Tubercle bacteria in sputum (carbol-fuchsin and methylene-blue).

(*e*) Stain in hot (steaming but not boiling) Ziehl's carbol-fuchsin for five minutes or in the cold solution for twenty minutes.

(*f*) Wash rapidly in water.

(*g*) Decolorize in 20 per cent. sulphuric, hydrochloric, or nitric acid, for three to five seconds.

(*h*) Wash in water.

(*i*) Contrast-stain in saturated aqueous solution of methylene-blue or in Löffler's blue for one-half minute.

(*j*) Wash in water, dry, and mount.

(*k*) Examine with the $\frac{1}{12}$ inch oil-immersion lens. The bacteria should be red in a blue field.¹

(2) **Koch-Ehrlich Method.**

(*a*) Stain cover-glass preparations in anilin fuchsin or anilin gentian-violet for twelve to twenty-four hours.

(*b*) Decolorize for three to five seconds in 20 per cent. nitric acid.

(*c*) Wash in water, in 60 per cent. alcohol for five to ten seconds, and again in water.

(*d*) Contrast-stain in aqueous methylene-blue for one minute.

(*e*) Wash, dry, and mount.

(3) **Gabbet's Method.**

(*a*) Stain in steaming Ziehl's carbol-fuchsin for one minute.

(*b*) Wash in water for two to three seconds.

(*c*) Stain in Gabbet's blue² for thirty seconds or longer.

(*d*) Wash in water, dry, and mount.

(4) **Rosenberger's Method.**

(*a*) Stain in carbol-fuchsin (cold) for five to ten minutes.

(*b*) Without washing stain for one to two minutes

¹ For the detection of tubercle bacteria in sputum when present in very small numbers see page 157.

² See Appendix, page 172.

in sweet spirits of nitre to which has been added enough alcoholic solution of malachite-green, Bismarck-brown, or methylene-blue to give a deep-colored fluid.

(*c*) Wash in water, dry, and mount.

3. Inoculations.

(*a*) Inoculate a guinea-pig subcutaneously in the abdominal wall with tuberculous material.

FIG. 89.—Tubercle bacteria in sputum (Fränkel and Pfeiffer).

(*b*) After four to six weeks,¹ when the inguinal lymphatic glands have become enlarged, kill the animal.

(*c*) With proper aseptic precautions make three or four cultures on blood-serum from two or three

¹ For class-work such an animal must be previously inoculated.

glands, spreading a large quantity of the material on the surface of the tubes.

(*d*) Seal air-tight, place in the incubator, and examine the growth that occurs.

4. Sections.

(*a*) Prepare sections of tubercular lesions from the inoculated animals or from the human lung in acute phthisis.

(*b*) Stain by the Ziehl-Neelson, Koch-Ehrlich, or Rosenberger methods.

5. Detection of Tubercle Bacteria in Urine.

(*a*) Make smears from the deepest layer of sediment thrown down by the centrifuge, or in the sedimenting glass.

(*b*) Stain in hot (steaming but not boiling) Ziehl's carbol-fuchsin for one minute.

(*c*) Wash in water.

(*d*) Decolorize in 20 per cent. sulphuric acid until pink.

(*e*) Wash in water, 95 per cent. alcohol for thirty seconds, and again in water.

(*f*) Stain in Löffler's methylene-blue for twenty seconds.

(*g*) Wash in water, dry, and mount.

The smegma bacillus, which frequently occurs in urine, does not stain by this method, as it is decolorized by alcohol. Rosenberger's method also gives excellent results with urine sediment.

6. Detection of Tubercle Bacteria in Milk.

(1) First Method.¹

(a) To 50 c.c. of suspected milk add 10 c.c. of carbolic acid.

(b) Shake vigorously for two to five minutes. Pour into a sedimenting glass, cover, and allow to stand for twenty-four hours ; or use a centrifuge.

(c) With a pipet remove the deepest layer of sediment and prepare films.

(d) Dry and pass three times through flame.

(e) Pass through equal parts of ether and alcohol.

(f) Dry and pass three times through the flame, and stain as directed for sputum.

(g) Mount and examine with the $\frac{1}{2}$ inch oil-immersion lens.

(2) Second Method.

(a) To 20 c.c. of the milk add 1 c.c. of a 50 per cent. potash solution.

(b) Heat in boiling water until the mixture turns yellowish brown.

(c) Add 20 c.c. of acetic acid, shake, heat again for three minutes, and centrifuge.

(d) Wash the sediment with hot water, again centrifuge, and make cover-glass preparations from the second sediment.

(e) Stain as directed for sputum.

¹This method may also be applied to the detection of tubercle bacteria in sputum when present in very small numbers, using 10-15 c.c. of sputum, 10 c.c. of water, and 6 c.c. of carbolic acid.

X. ACTINOMYCOSIS.

1. Study the morphology and biology of cultures of *Actinomyces bovis*.
2. Prepare sections from tissue containing colonies of the parasite.
3. Stain by Gram's method or as follows :

FIG. 90.—*Actinomyces* (von Jaksch).

- (a) Stain deeply in saturated aqueous eosin solution for ten minutes.
- (b) Wash in water.
- (c) Stain in anilin-gentian-violet for two to five minutes.
- (d) Wash in normal salt solution.¹
- (e) Treat with iodine solution (iodine, 1 part ; potassium iodid, 2 parts ; distilled water, 100 parts), for one minute.
- (f) Wash in water and dry slightly with filter-paper.

¹ See Appendix, page 173.

(*g*) Clear in anilin oil, then in several changes of xylol.

(*h*) Mount in balsam.

XI. MALARIA.

1. Examination of Fresh Blood for the Malarial Hematozoon.

(*a*) Clean a slide and cover-glass.

(*b*) Place a small drop of the blood to be examined on the slide, cover, and seal with vaselin.

(*c*) Examine with the oil-immersion lens.

The ameboid movements of the Hematozoon malariae are visible at the room-temperature, but become more active if the slide is warmed.

2. Stained Preparations.

(1) First Method.

(*a*) Place a small drop of blood between two cover-glasses, slide the covers apart, and allow the films to dry.

(*b*) Fix by placing in absolute alcohol for five minutes or in the vapor of formaldehyd for two and a half minutes, or by one of the methods given under Anthrax (page 131).

(*c*) Stain in a 1 per cent. aqueous solution of eosin for five minutes or, if formaldehyd is used for fixation, in an alcoholic (60–75 per cent.) solution of eosin for three to ten minutes.

(*d*) Contrast-stain in saturated aqueous solution of methylene-blue for five minutes for the alcoholic preparations, for one minute for the formaldehyd

FIG. 91.—Some of the principal forms assumed by the Hematozoon of tertian fever in the course of its cycle of development (after Thayer and Hewetson).

preparations, or in Ehrlich-Biondi fluid¹ for one-half hour.

FIG. 92.—Crescentic and flagellated forms of Hematozoon malariae: 1, flagellated form of tertian fever; 2, flagellated form of quartan fever; 3, crescents, and 4, flagellated form of estivo-autumnal fever (after Thayer and Hewetson).

¹ See Appendix, page 173.

(e) Wash, dry, and mount. Ehrlich-Biondi preparations are examined dry.

In these preparations the red corpuscles are stained red, the malarial organisms and the nuclei of the leukocytes blue.

(2) **Whitney's Method.**

(a) Spread the blood as usual ; dry thoroughly in the air or by gentle heat.

(b) Treat for twenty seconds with the following modification of Zenker's fluid :

Potassium bichromate,	2 gm. ;
Sodium sulphate,	1 gm. ;
Water,	100 c.c.

Saturate while warm with corrosive sublimate.

Add 5 per cent. of strong nitric acid at time of using.

(c) Wash in water, and dry with filter-paper.

(d) Cover with Ehrlich's triacid stain¹ for three minutes.

(e) Wash, dry, and mount.

Unna's polychrome methylene-blue² and the Chenzinsky-Plein eosin and methylene-blue³ solution work well after this fixation, but not Löffler's methylene-blue.

¹ See Appendix, page 172.

² See Appendix, page 172.

³ See Appendix, page 173.

APPENDIX.

I. BACTERIAL MEASUREMENTS BY PHOTOGRAPHY.

Wilson and Randolph¹ recommend the following procedure for accurately measuring bacteria :

(a) Prepare a drawing about four times the size of the desired negative by ruling with ink two sets of equidistant lines at right angles to each other, making every tenth line somewhat heavier than the others.

(b) Reduce this drawing by photography to such a size that the rulings are exactly 1 millimeter apart.

(c) Adjust the photographic apparatus so that an amplification of 1000 diameters is secured. Obtain this by measuring the image of a stage micrometer on the ground-glass screen.

(d) With this adjustment make a photomicrograph of the bacteria to be measured.

(e) Superimpose the print of the photomicrograph of the bacteria on the print of the scale, or

¹ *Journal of Applied Microscopy*, vol. ii, No. 11, Nov., 1899.

vice versa. The reading is directly in micromillimeters or μ .

FIG. 93.—Wilson and Randolph's method of measuring bacteria by photography.

II. MOULDS AND YEASTS.

Moulds and yeasts frequently contaminate plate and tube cultures, and inasmuch as these growths are occasionally the cause of pathologic conditions,

FIG. 94.—*Saccharomyces cerevisiæ*.

it is advisable that the bacteriologist be acquainted with certain typical forms.

FIG. 95.—*Mucor racemosus*: *a*, spore-bearing head; *b*, spores; *c*, branch; *d*, resting spores (after Jelliffe).

1. *Saccharomyces cerevisiæ*.

Examine cultures of this yeast, mount, and stain exactly as directed for bacteria.

2. *Mucor racemosus*.

(1) Cultures of this mould may be made on the surface of gelatin- or agar-tubes, but preferably in plates. The developing colonies may be examined directly with the low-power lens of the microscope.

FIG. 96.—*Aspergillus repens*: *a*, conidia-bearing head; *b*, conidia; *c*, peritheca; *d*, sterigmata (after Jelliffe).

(2) For examination with the high power, remove a small portion of the growth to a mixture of glycerin and water on a slide; spread out as thin as possible, cover, and examine.

(3) The preparation may be stained by adding a little eosin solution to the glycerin and water.

(4) Permanent preparations may be made as follows:

(*a*) Transfer a small amount of the mould to a slide.

(b) Drop a little alcohol upon it to remove the air from the hyphæ.

FIG. 97.—*Penicillium crustaceum* : *a*, conidia with mycelium ; *b*, conidia cluster ; *c*, sterigma ; *d*, conidia (after Jelliffe).

(c) Treat again with water.

(d) Stain in methylene-blue.

(e) Mount in glycerin or glycerin-jelly.

3. *Aspergillus repens* and *Penicillium crustaceum*.

These moulds may be examined exactly as directed for *Mucor*.

4. Key for the Identification of the Yeasts and Moulds that most frequently contaminate Cultures.¹

Mycelium growth absent:

I. YEASTS, SACCHAROMYCETACEÆ.

1. Growth white to dirty white,
S. cerevisiæ.
S. albicans.
2. Growth reddish,
S. glutinis.
3. Growth brownish to black,
S. niger.

Mycelium growth present:

II. MOULDS.

- I. Spores inside of sporangia.

PHYCOMYCETES. Family, MUCORINI.

- (a) Mycelium of one kind,

1. Spore-bearing bodies on unbranched hyphæ,

Mucor.

2. Spore-bearing bodies on branched hyphæ,

Circinella.

- (b) Mycelium of two kinds,

Rhizopus.

¹ Adapted from "Some Laboratory Moulds," by S. E. Jelliffe, in *Journal of Pharmacology*, Nov., 1897.

2. Spores free, at the ends of modified hyphæ,
HYPHOMYCETES.

(a) Hyphæ pallid, loose, not collected into
fascicles,

MUCEDINEÆ.

1. Conidia undivided,

a. Hyphæ short,

(1) Hyphæ unbranched,

Oospora.

(2) Hyphæ branched,

Monilia.

b. Hyphæ elongated,

(1) Conidia aggregated,

* Fertile hyphæ enlarged at apex,

† Conidia on simple sterigmata.

Aspergillus.

†† Conidia on compound sterigmata,

Sterigmatocystis.

** Fertile hyphæ not enlarged at apex,

Penicillium.

(2) Conidia separated or loosely aggregated,

Botrytis.

2. Conidia once septate,

Cephalothecium.

(b) Hyphæ brownish or black, not collected into
fascicles,

DEMATIÆ.

1. Conidia non-septate,

a. Hyphæ short, only slightly different
from conidia,

Torula.

The watery solutions soon decompose, and must be made only as required for use. Methylene-blue, however, may be made up as a saturated aqueous solution, as it is permanent. Always filter a stain before use.

2. Kuhne's Methylene-blue.

Methylene-blue,	1.5 gm. ;
Absolute alcohol,	10 c.c. ;
Carbolic acid (1 : 20),	100 c.c.

Stain films five minutes.

3. Löffler's Methylene-blue.

Saturated alcoholic solution of methylene-blue,	30 c.c. ;
Solution of potassium hydrate in water (1 : 10,000),	100 c.c.

4. Carbol-thionin-blue.

Thionin-blue,	1 gm. ;
Carbolic acid (1 : 40),	100 c.c.

Dilute 1 volume of the stain with 3 of water, when required for use. Stain from three to five minutes.

5. Anilin-water Solution.

Anilin oil,	5 c.c. ;
Water,	100 c.c.

Shake together, allow to stand for five minutes, and filter through a moistened filter.

6. Anilin-gentian-violet and Anilin-fuchsin.

Anilin-water,	10 parts ;
Saturated alcoholic solution of gentian-violet or fuchsin,	1 part.

7. Gram's Iodin Solution.

Iodin,	1 part;
Potassium iodid,	2 parts;
Distilled water,	300 "

8. Ziehl's Carbol-fuchsin.

Basic fuchsin,	1 part;
Absolute alcohol,	10 parts;
Carbolic acid (1 : 20),	100 "

9. Gabbet's Blue.

Sulphuric acid (25 per cent. solution),	100 c.c.;
Methylene-blue,	2 gm.

Allow the diluted acid to stand twenty-four hours or until it is cold before adding the methylene-blue.

10. Unna's Polychrome Methylene-blue.

Methylene-blue,	1 part ;
Potassium carbonate,	1 "
Water,	100 parts.

Must be ripened for months. The ripened solution may be procured from Grüber.

11. Ehrlich's Triacid Stain.

Saturated aqueous solution of orange G,	120 parts.
Acid fuchsin,	80 "
Methyl-green,	100 "
Distilled water,	300 "
Absolute alcohol,	180 "
Glycerin,	50 "

Never shake the solution. Pipet from the top what is needed for use.

12. Ehrlich-Biondi Stain.

Is best procured ready made from Grübler.

13. Chenzinsky-Plein Stain.

Saturated aqueous solution of	
methylene-blue,	40 c.c. ;
Alcoholic (70 per cent.) solu-	
tion of eosin (1 : 200),	20 c.c. ;
Distilled water,	40 c.c.

The best results are obtained by staining for twenty-four hours. Fairly good results may be obtained by using warmed stains for fifteen minutes.

14. Normal Salt Solution.

Distilled water,	100 c.c. ;
Sodium chlorid,	0.75 gm.

15. Acid Alcohol.

Alcohol (70 per cent.),	97 c.c. ;
Hydrochloric acid,	3 c.c.

16. Zenker's Fluid.

Potassium bichromate,	2.5 gm. ;
Sodium sulphate,	1 gm. ;
Corrosive sublimate,	5 gm. ;
Glacial acetic acid,	5 c.c. ;
Water,	<i>ad</i> 100 c.c.

Do not add the acetic acid until ready for use.

Fix tissues from one to twenty-four hours.

Wash in running water from twelve to twenty-four hours. Preserve in 80 per cent. alcohol.

17. Cleaning Mixture, for Slides, Cover-glasses, and Glassware.

Potassium bichromate,	6 gm.;
Sulphuric acid,	6 c.c.;
Water,	100 c.c.

Wash in water and alcohol.

18. Nessler's Solution.

A. Potassium iodid,	35 gm.;
Water,	200 c.c.
B. Mercuric chlorid,	16 gm.;
Water,	500 c.c.

Add B to A until faint show of excess is indicated, then add 160 grams of solid potassium hydrate. Dilute to 1 liter, and add strong solution of mercuric chlorid, little by little, until the red mercuric iodid just begins to be permanent. Do not filter. Solution should be pale straw-color. It is improved by age.

19. Phenol-sulphonic Acid.

Sulphuric acid (pure and concentrated),	148 c.c.;
Distilled water,	12 c.c.;
Pure carbolic acid,	24 gm.

IV. TABLE OF SYNONYMS.

The scientific names of species used throughout this work are those adopted by Migula. They are preferable because they conform to the laws of scientific nomenclature and priority. The following table gives the synonyms of many of the common species in alphabetic order:

BACILLUS—

- albolactus, Mig.; *B. lactis albus*, Löffler.
 amylovorus (Burrill), De Toni ; *M. amylovorus*, Burrill.
 atterimus, L. et N.; *B. mesentericus niger*, Lunt.
 avium, Mig.; *B. diphtheriæ avium*, Kruse.
 chauvæi, Arloing, Cornevin et Thomas ; *B. carbonis*,
 Mig.; *B. anthracis symptomatici*, Kruse.
 coli, Mig.; *Bact. coli commune*, Escherich.
 diaphanus, Mig.; *Halibacterium pellucidum*, Fischer.
 dysenteriæ (Kruse), Mig.; *B. dysenteriæ liquefaciens*,
 Kruse.
 equi, Mig.; *B. equi intestinalis*, Dyar et Keith.
 fischeri, Mig.; *Photobacterium fischeri*, Beyerinck ; *B.*
phosphorescens indigenus, Kruse.
 globigii, Mig.; *B. mesentericus ruber*, Globig.
 indicus, Koch ; *B. indicus ruber*, Mig.
 intricatus, Mig.; *Cladothrix intricata*, Russell.
 limnophilus, Mig.; *B. limosus*, Russell.
 mesentericus, Mig.; *B. mesentericus fuscus*, Flügge.
 mirabilis, Mig.; *Proteus mirabilis*, Hauser.
 murium, Mig.; *B. typhi murium*, Löffler.
 niger, Mig.; *B. lactis niger*, Gorini.
 ozænæ, Mig.; *B. foetidus ozænæ*, Hajek.
 phosphorescens, Fischer ; *Photobacterium indicum*, Beyerinck ; *B. phosphorescens indicus*, Kruse.

BACILLUS—

- phosphoricus, Mig.; *B. argenteo-phosphorescens* III., Katz.
- prodigiosus, Flügge; *Monas prodigiosa*, Ehrenberg; *Bact. prodigiosum*, Schröter; *M. prodigiosus*, Cohn.
- pseudotuberculosis, Mig.; *B. pseudotuberculosis liquefaciens*, Kruse.
- suipestifer, Kruse; *B. of hog-cholera*, Salmon-Smith; *B. cholerae suum*, Mig.
- sulfureus, Mig.; *Proteus sulfureus*, Lindenborn.
- typhosus, Mig.; *B. typhi abdominalis*, Mig.
- vulgaris, Mig.; *Proteus vulgaris*, Hauser.
- vulgatus, Mig.; *B. mesentericus vulgatus*, Flügge.
- zenkeri, Hauser; *Proteus zenkeri*, Hauser.
- zopfii, Mig.; *Bact. zopfii*, Kurth.

BACTERIUM—

- aceti, Zopf; *Ulvina aceti*, Kützing; *Mycoderma aceti*, Thomsen.
- acidi lactici, Mig.; *B. acidi lactici* I., Hüppe.
- aërogenes, Mig.; *Bact. lactis aërogenes*, Escherich.
- amethystinum, Mig.; *B. membranaceus amethystinus*, Eisenberg.
- anthracis, Mig.; *B. anthracis*, Koch.
- anthracoides, Mig.; *B. anthracoides*, Hüppe et Wood.
- aquatile, Mig.; *B. aquatile*, Frankland.
- arborescens, Mig.; *B. arborescens*, Frankland.
- aurescens, Mig.; *B. aurescens*, Frankland.
- bienstockii, Schröter; *B. coprogenes parvus*, Bienstock.
- bovis, Mig.; *Pneumobacillus liquefaciens bovis*, Arloing; *B. pneumonicus liquefaciens*, Kruse.
- brunneum, Mig.; *B. brunneus*, Adametz-Wichmann.
- buccale, Mig.; *Leptothrix buccalis*, Robin.
- candicans, Mig.; *B. candicans*, Frankland.

BACTERIUM—

- capsulatum, Pfeiffer ; *B. capsulatus*, Pfeiffer, Koch.
carotarum, Mig.; *B. carotarum*, Koch.
chlorinum, Mig.; *B. chlorinus*, Frankland.
citreum, Mig.; *B. citreus*, Frankland.
cœruleum, Mig.; *B. cœruleus*, Smith.
columbarum, Mig.; *B. diphtheriæ columbarum*, Löffler.
coprogenes, Mig.; *B. coprogenes foetidus*, Flügge.
cuniculicida, Koch ; *B. cholerae gallinarum*, Flügge ;
Bact. septichæmiæ, Schröter.
cuniculi, Mig.; *B. cuniculi pneumonicus*, Kruse.
endocarditidis, Mig.; *B. endocarditidis capsulatus*, Weichselbaum.
felis, Mig.; *B. felis septicus*, Kruse.
giardi, Mig.; *B. phosphorescens giardi*, Kruse.
keratomalaciæ, Mig.; *B. septicus keratomalaciæ*, Babes.
lacteum, Mig.; *B. lactis III.*, Kruse.
lacticum, Mig.; *B. lacticus*, Kruse ; *B. lactis acidi*, Liebmänn.
laerii, Mig.; *B. viscosus I.*, van Laer ; *B. viscosus cerevisiæ*, Kruse.
lepræ, Mig.; *B. lepræ*, Hansen.
mallei, Mig.; *B. mallei*, Löffler.
murisepticum, Mig.; *B. murisepticus*, Flügge ; *B. murinus*, Schröter.
mycoides, Mig.; *B. mycoides roseum*, Scholl-Holschewnikoff.
nephritidis, Mig.; *B. nephritidis interstitialis*, Letzerick.
palumbarium, Mig.; *B. cholerae columbarum*, Kruse.
phosphorescens, Fischer ; *Photobacterium phosphorescens*, Beyerinck.
proteus, Mig.; *Proteus capsulatus septicus*, Banti ; *B. capsulatus septicus*, Kruse.

BACTERIUM—

- pseudodiphthereticum, Mig.; *Corynebacterium pseudodiphthereticum*, L. et N.
 pseudoinfluenzæ, Mig.; *B. pseudoinfluenzæ*, Kruse.
 pseudotuberculosis, Mig.; *B. pseudotuberculosis*, Pfeiffer ;
Streptobacillus pseudotuberculosis rodentium, Preisz.
 pyocinnabarium, Mig.; *B. pyocinnabarius*, Kruse.
 pyogenes, Passet ; *B. pyogenes foetidus*, Passet.
 rhusiopathiæ, Mig.; *B. rhusiopathiæ suis*, Kitt ; *Bact. erysipelatus suis*, Mig.
 salivæ, Mig.; *B. salivæ minutissimus*, Kruse.
 sempervivum, Mig.; *B. lactis XII.*, Kruse.
 smegmatis, Mig.; *B. smegmatis*, Kruse.
 subtile, Mig.; *B. subtilis simulans I.*, Bienstock ; *B. faecalis I.*, Kruse.
 suicida, Mig.; *B. suisepcticus*, Kruse.
 termophilum, Mig.; *B. termophilus*, Miquel.
 tuberculosis avium, Mig.; *B. tuberculosis avium*, Maffucci ; *Mykobacterium tuberculosis avium*, L. et N.
 tuberculosis, Mig.; *B. tuberculosis*, Koch ; *Mykobacterium tuberculosis*, L. et N.
 ubiquitum, Mig.; *B. ubiquitus*, Jordan.
 varicosum, Mig.; *B. varicosus conjunctivæ*, Hombert.
 vermiculare, Mig.; *B. vermicularis*, Frankland.
 vaginali, Mig.; *B. g.*, Vignal ; *B. buccalis minutus*, Sternberg.
 welchii, Mig.; *B. aërogenes capsulatus*, Welch.
 xerosis, Mig.; *B. xerosis*, Neisser et Kuschbert.

MICROCOCCUS—

- albocereus, Mig.; *Staphylococcus cereus albus*, Passet.
 amplus, Mig.; *M. albicans amplus*, Flügge.
 aurantiacus, Cohn ; *Staphylococcus cereus aureus*, Mig.

MICROCOCCUS—

- aureus, Mig.; *Staphylococcus pyogenes aureus*, Rosenbach; *M. pyogenes aureus*, Mig; *M. pyogenes*, L. et N.
cereus, Mig.; *Staphylococcus cereus flavus*, Passet.
citreus, Mig.; *Staphylococcus pyogenes citreus*, Passet.
citrinus, Mig.; *Diplococcus citreus liquefaciens*, Unna.
conglomeratus, Flügge; *M. citreus conglomeratus*, Flügge; *Diplococcus citreus conglomeratus*, Eisenberg.
conjunctivitis, Mig.; *M. flavus conjunctivæ*, Gombert.
conoideus, Mig.; *Staphylococcus salivarius pyogenes*, Biondi.
corrugatus, Mig.; *Merismopedia mesenterica corrugata*, Dyar.
coryzæ, Mig.; *Diplococcus coryzæ*, Hajek.
cuniculorum, Mig.; *M. pyæmiæ cuniculorum*, Schröter.
cyaneus, Cohn; *Bacteridium cyaneum*, Schröter.
desidens, Mig.; *M. flavus desidens*, Flügge.
faviformis, Mig.; *M. lacteus faviformis*, Flügge.
fragilis, Mig.; *Merismopedia fragilis*, Dyar.
gonorrhœæ, Flügge; *Gonococcus*, Neisser.
intracellularis, Mig.; *Diplococcus intracellularis meningitidis*, Weichselbaum; *Streptococcus intracellularis*, L. et N.
lacticus, Mig.; *Sphærococcus acidi lactici*, Marpmann.
liquefaciens, Mig.; *M. ureæ liquefaciens*, Flügge.
luteus, Cohn; *Bacteridium luteum*, Schröter.
mollis, Mig.; *Merismopedia mollis*, Dyar.
phosphoreus, Cohn; *M. lucens*, v. Tieghem; *M. pflügeri*, Ludwig, ex parte; *Photobacterium phosphorescens*, Beyerinck.
pyogenes, Mig.; *Staphylococcus pyogenes albus*, Rosenbach; *M. pyogenes albus*, L. et N.
roseus, Flügge; *Diplococcus roseus*, Eisenberg.

MICROCOCCUS—

- rugatus, Mig.; *M. endocarditidis rugatus*, Weichselbaum.
 salivarius, Mig.; *Coccus salivarius septicus*, Biondi.
 stellatus, Frankland; *Coccus stellatus*, Lustig.
 subflavidus, Mig.; *M. tetragenus subflavus*, v. Besser.
 tardigradus, Mig.; *M. flavus tardigradus*, Flügge.
 tardior, Mig.; *Diplococcus flavus liquefaciens tardus*, Unna et Tommasoli.
 tardissimus, Mig.; *Diplococcus albicans tardissimus*, Flügge.
 tardus, Mig.; *Diplococcus albicans tardus*, Unna et Tommasoli.
 tenuis, Mig.; *M. pyogenes tenuis*, Rosenbach.
 tenuissimus, Mig.; *M. cumulatus tenuis*, v. Besser.
 varians, Mig.; *Merismopedia flava varians*, Dyar.

MICROSPIRA—

- annularis, Mig.; *Photobacterium annulare*, Fischer.
 canalis, Mig.; *Vibrio saprophiles* γ , Weibel.
 caraibica, Mig.; *Photobacterium caraibicum*, Fischer.
 comma, Schröter; *Spirillum cholerae asiaticæ*, Flügge;
Vibrio cholerae asiaticæ, Mig.; *Vibrio comma*, Mig.
 coronata, Mig.; *Photobacterium coronatum*, Fischer.
 degenerans, Mig.; *Photobacterium degenerans*, Fischer.
 delgadensis, Mig.; *Photobacterium delgadense*, Fischer.
 dunbari, Mig.; *Vibrio dunbari*, Mig.
 finkleri, Schröter; *Spirillum finkleri*, Mig.; *Vibrio finkleri*, Mig.; *Vibrio proteus*, Mig.
 glutinosa, Mig.; *Photobacterium glutinosum*, Fischer.
 luminosa, Mig.; *Photobacterium luminosum*, Fischer.
 marina, Mig.; *Spirillum marinum*, Russell.
 metschnikovi, Mig.; *Vibrio metschnikovi*, Gamaleïa.
 papillaris, Mig.; *Photobacterium papillare*, Fischer.

MICROSPIRA—

saprophiles, Mig.; *Vibrio saprophiles*, β , Weibel.
 tonsillaris, Mig.; *Vibrio tonsillaris*, Klein.
 tuberosa, Mig.; *Photobacterium tuberosum*, Fischer.

PSEUDOMONAS—

æruginea, Mig.; *Bact. æruginosum*, Schröter; *B. æruginosus*, Schröter; *B. pyocyaneus*, Gessard.
alba, Mig.; *B. fluorescens albus*, Zimmermann; *B. fluorescens non-liquefaciens* (?), Eisenberg.
eisenbergii, Mig.; *B. fluorescens non-liquefaciens*, Eisenberg
europæa, Mig.; *Nitrosomonas europæa*, Winogradsky.
fluorescens, Mig.; *B. fluorescens liquefaciens*, Flügge.
ianthina, Mig.; *Bact. ianthinum*, Zopf; *B. janthinus*, Zimmermann.
javanica, Mig.; *Photobacterium javanense*, Eijkmann.
javaniensis, Mig.; *Nitrosomonas javaniensis*, Winogradsky.
laurentia, Mig.; *B. violaceus laurentius*, Jordan.
litoralis, Russell; *B. litoralis*, Russell.
minutissima, Mig.; *B. fluorescens liquefaciens minutissimus*, Unna et Tommasoli.
pseudianthina, Mig.; *B. violaceus*, Frankland.
tenuis, Mig.; *B. fluorescens tenuis*, Zimmermann.

SARCINA—

ventriculi, Goodsir; *Merismopedia goodsiri*, Husem;
Merismopedia ventriculi, Robin; *Sarcina fuscescens*, de Bary.

SPIROSOMA—

attenuatum, Mig.; *Spirillum attenuatum*, Warming.
flavescens, Mig.; *Vibrio flavescens*, Weibel.

SPIROSOMA—

flavum, Mig.; *Vibrio flavus*, Weibel.

gregarium, Mig.; *Myconostoc gregarium*, Cohn.

STREPTOCOCCUS—

cerasinus, Mig.; *M. cerasinus siccus*, List.

citreus, Mig.; *M. citreus*, List-Eisenberg.

cystitidis, Mig.; *Diplococcus ureæ pyogenes*, Rovsing.

equi, Schütz; *S. coryzæ contagiosæ equorum*, Eisenberg.

giganteus, Mig.; *S. giganteus urethræ*, Lustgarten et Mannaberg.

gracilis, Mig.; *S. coli gracilis*, Escherich.

mastitidis, Guillebeau; *S. mastitidis sporadicæ*, Guillebeau; *S. agalactiæ contagiosæ*, Kitt.

pyogenes, Rosenbach; *S. erysipelatos*, Rosenbach; *S. conglomeratus*, Kurth; *S. brevis*, v. Lingelsheim; *S. longus*, v. Lingelsheim; *S. murisepticus*, v. Lingelsheim; *S. septo-pyæmicus*, Biondi.

septicus, Mig.; *S. septicus liquefians*, Babes; *S. septicus liquefaciens*, Babes.

INDEX.

- ACID alcohol, 173
 carbolic, 44, 124
 phenol-sulphonic, 94, 174
 production, 94
 white, 55
 Acidity, determination of, 52
 relation to, of medium, 91
 Actinomyces bovis, 158
 Actinomycosis, 158
 Adhesive preparations of colonies, 75
 Aërobic organisms, 75, 76, 78
 Agar culture-medium, 51
 cultures, 61
 Air, analysis of, 121
 bacteria in, 121
 Algæ, brown, 40
 green, 40
 red, 40
 Alkalinity, determination of, 52
 relation to, of medium, 91
 Alkali production, 94
 Amebabacteriaceæ, 42
 Amebacter, 42
 American Public Health Association, 52, 83, 85
 Ammonia, production of, 92
 test for, 93
 Amphitrichous, 25
 Anaërobic cultures, 78
 organisms, 75, 76, 78, 80
 Analysis of air, 121
 of milk, 120
 of soil, 122
 of water, qualitative, 115
 quantitative, 114
 Anilin fuchsin, 171
 gentian-violet, 171
 stains, 18
 water, 171
 Anthrax, 130
 Antiseptics, 44
 Arnold's steam sterilizer, 46, 47, 48
 Arrangement of bacteria, 85
 Asiatic cholera, 27, 64
 Aspergillus repens, 166, 168
 Autoclave, 46, 49
 Autopsy, making, 126
 BACILLI, form of, 22
 groups of, 109
 reproduction of, 34
 species of, 102
 Bacillus, 39, 102, 109
 acidì lactici I., 176
 aërogenes capsulatus, 178
 aëruginosus, 180
 anthracis, 176
 symptomatici, 175
 anthracoides, 176
 aquatile, 176
 arborescens, 176
 argenteo-phosphorescens, III., 176
 aurescens, 176
 brunneus, 176
 buccalis minutus, 178
 candicans, 176
 capsulatus, 176
 septicus, 177
 carbonis, 175
 carotarum, 177
 chauvæi, 38
 chlorinus, 177
 cholerae columbarum, 177
 gallinarum, 177
 suum, 176
 citreus, 177
 cloacæ, 115
 cœruleus, 117
 coli, colony of, 146
 for removing sugars, 56
 in water, 115
 isolation of, 119, 120
 study of, 144
 test for, 118
 coli communis, 175
 coprogenes fœtidus, 177

- Bacillus coprogenes parvus*, 176
cuniculi pneumonicus, 177
diphtheriæ, 136, 137, 138
 avium, 175
 columbarum, 177
dysenteriæ liquefaciens, 175
endocarditidis capsulatus, 177
equi intestinalis, 175
fæcalis I., 178
felis septicus, 177
fluorescens albus, 181
 liquefaciens, 181
 minutissimus, 181
 non-liquefaciens, 181
 tenuis, 181
fœtidus ozænæ, 175
g, Vignal, 178
indicus ruber, 175
janthinus, 181
lacticus, 177
lactis III., 177
 XII., 178
 acidi, 177
 albus, 175
 niger, 175
lepræ, 177
limosus, 175
litoralis, 181
mallei, 177
membranaceus amethystinus, 176
mesentericus, 63
 fuscus, 175
 niger, 175
 ruber, 175
 vulgatus, 176
murinus, 177
murisepticus, 177
muscoïdes, 74
mycoïdes, 63
 roseum, 177
nephritidis interstitialis, 177
œdematis, 63
of hog cholera, 176
phosphorescens giardi, 177
 indicus, 175
 indigenus, 175
pneumonicus liquefaciens, 176
polypiformis, 69
prodigiosus, 80
pseudoinfluenzæ, 177
pseudotuberculosis, 178
 liquefaciens, 176
pyocinnabarius, 178
pyocyaneus, 180
pyogenes fœtidus, 178
Bacillus radiatus, 63, 69
 rhusiopathiæ suis, 178
 salivæ minutissimus, 178
 septicus keratomalaciæ, 177
 smegmatis, 152, 156, 178
 sporogenes, 115
 subtilis, 19, 35
 simulans I. 178
 suipestifer, 24
 suisepcticus, 178
 syphilidis, 152
 termophilus, 178
 tuberculosis, 178
 avium, 178
typhi abdominalis, 176
 murium, 175
typhosus, colony of, 146
 gelatin culture, 63
 in milk, 120
 in water, 115
 isolation of, 115
 showing flagella, 28, 147
 study of, 144
 Widal reaction, 145
ubiquitus, 178
varicosus conjunctivæ, 178
vermicularis, 178
violaceus, 181
 laurentius, 181
viscosus I., 177
 cerevisiæ, 177
vulgaris, 19, 115
xerosis, 178
Bacteriaceæ, 39, 99
Bacteria, classification of, 39, 99
 by groups, 107
 cultures of, 60
 form of, 22
 groups of, 107
 in air, 121
 in milk, 120
 classification of, by groups, 112, 121
 in soil, 122
 in tissues, 20
 in water, 114
 classification of, by groups, 112
 measurement of, 11, 163
 morphology of, 22
 pathogenic, 124
 reproduction of, 34
 species of, 99
Bacteridium cyaneum, 179
 luteum, 179
Bacteriologic committee, 52, 83, 85

- Bacterio-purpurin, 39, 41
 Bacterium, 39, 99, 107
 aërogenes, 115
 aëruginosum, 180
 anthracis, colony of, 74, 77, 131
 gelatin culture, 63
 showing spores, 36
 study of, 130, 132, 133
 coli commune, 175
 diphtheriæ, 135, 136, 137, 138
 diagnosis of, 135, 138
 isolation of, 137
 slide for examination of, 140
 test for virulence, 139
 erysipelatos suis, 178
 faschingii, 30
 ianthinum, 181
 influenzæ, 141, 142, 143
 lactis aërogenes, 176
 lepræ, 152
 mallei, 133, 134
 parvum, 69
 pneumoniæ, 32, 150
 pneumonicum, 150, 151
 prodigiosum, 176
 septicæmiæ, 177
 smegmatis, 152, 156, 178
 tuberculosis, 76, 151
 zopfii, 176
 Basic fuchsin, 18, 170
 Bath, paraffin and water, electric, 68
 Beef, Liebig's extract of, 55
 Beggiatoa, 41
 Beggiatoaceæ, 41
 Berkefeld filter, 45
 Binary division, 34
 Biologic characters of a species, 83, 84
 examination of water, 114
 Bismarck brown, 20, 170
 Blood agar, 141
 examination in anthrax, 131
 in malaria, 159, 161
 Whitney's method, 161
 preparations, 131, 159, 161
 Blood-serum cultures, 66
 Löffler's, 58
 tubes, 58
 Boni's method for capsules, 33
 Boston Board of Health sterilizer, 47
 Bouillon, 51
 cultures, 60
 dextrose, 56
 Bouillon, lactose, 56
 saccharose, 56
 Brown algæ, 40
 Brownian movement, 24, 25
 Brown University paraffin and water bath, 68
 Bryophyta, 40
 Buchner's method for anaërobes, 78

 CAMERA lucida, 12
 Capsules, demonstration of, 31
 Carbohydrates, 92
 Carbol-fuchsin, Ziehl's, 172
 Carbolic acid, 44, 124
 Carbol-thionin blue, 171
 Cell, 15
 Chamberland filter, 45
 Characeæ, 40
 Chenzinsky-Plein stain, 173
 Chester, 95, 107
 Chester and Robin pipet, 147, 148
 Chlamydobacteriaceæ, 41
 Chlamydothrix, 41
 Chlorophyceæ, 40
 Cholera, Asiatic, 27, 64, 180
 Chromatiaceæ, 42
 Chromatium, 42
 Cladothrix intricata, 175
 Classification of bacteria, 39
 by groups, 107
 of dairy bacteria by groups, 112, 121
 of water bacteria by groups, 112
 Cleaning mixture, 14, 174
 Clostridium, 35
 Club mosses, 40
 Coccaceæ, 39, 95
 Cocci, 22
 Coccus salivarius septicus, 179
 stellatus, 179
 Colon bacillus, 144
 Colonies, counting, 116, 117, 118
 impression, or adhesive preparations of, 75
 Colony, 60
 Concave slide, 15
 Condenser, 11
 Conjugatæ, 40
 Conjugates, 40
 Conn, 112
 Contamination of water by sewage, 115
 Cornet's forceps, 17
 Corrosive sublimate solution, 44, 124

- Corynebacterium pseudodiphtheriticum*, 177
 Cotton, 48
 Cotton-wool filter, 45
 Counting-plate, Jeffer's, 117, 118
 Wolfhügel's, 116, 117
 Cover-glass forceps, 17
 Crenothrix, 41
 Cryptogamia, 40
 Culture-media, 51
 Cultures, agar, 61
 anaërobic, 78
 blood-serum, 66
 bouillon, 60
 gelatin, 61
 in the fermentation-tube, 76
 of bacteria, 60
 plate, 66
 potato, 66
 pure, 60
 stab, 61
 stroke, 64
- DAIRY bacteria, classification of, 112, 121
 Demonstration of arrangement, 85
 of capsules, 31
 of division, 34
 of flagella, 25
 of form, 22, 85
 of liquefying ferment, 80
 of motion, 24, 90
 of pleomorphism, 90
 of spores, 35, 90
 Determination of acid production, 94
 of acidity of media, 52
 of alkalinity of media, 52
 of alkali production, 94
 of arrangement, 85
 of form, 22, 85
 of motility, 24, 90
 of name, 95
 of pleomorphism, 90
 of species, 83
 of thermal death-point, 91
 Dextrose, 56
 Diagnosis of diphtheria, 135
 of glanders, 134
 of influenza, 141
 slide for diphtheria, 140
 Diaphragm, 11
 Diatomæ, 40
 Diatoms, 40
 Dinoflagellates, 40
- Diphtheria, 135
 diagnosis, 135
 slide, 140
 diagnostic stains, 138
 inoculations of, 140
 isolation of bacterium, 137
 outfit for diagnosis, 136
 test for virulence, 139
 Diplococcus, 22, 34
 albicans tardissimus, 180
 tardus, 180
 citreus conglomeratus, 179
 liquefaciens, 178
 coryzæ, 179
 flavus liquefaciens tardus, 180
 intracellularis meningitidis, 179
 roseus, 179
 ureæ pyogenes, 181
 Discontinuous sterilization, 43
 Disinfectant, 44
 Disinfection, 44
 Division, reproduction by, 34
 Drumstick bacillus, 33
- EHRLICH-BIONDI stain, 173
 Ehrlich's triacid stain, 172
 Electric paraffin and water bath, 63
 Endogenous spores, 34
 Eosin, 20
 Estivo-autumnal fever, 160
 Etched tubes, 55
 Eubacteria, 39
 Examination of gonorrhœal pus, 129
 of tuberculous sputum, 152
 Extract of beef, Liebig's, 55
 Eyepiece, 11
- FACULTATIVE anaërobic, 78
 Fermentation-tubes, 57
 cultures in, 76
 Ferment, liquefying, 80
 Ferns, 40
 Fever, estivo-autumnal, 160
 malarial, 159
 quartan, 160
 tertian, 160
 Filter, 45
 cotton-wool, 45
 for culture-media, 53
 Kitasato's, 46
 porcelain, 45
 Filtration, 45
 of blood-serum, 59
 of culture-media, 53
 Fission-algæ, 40

- Fission-fungi, 40
 Fission-plants, 40
 Fixing blood smears, 131, 159, 161
 Flagella, 25
 Flügge, 95
 Forceps, cover-glass, 17
 Form, 22, 85
 Formalin, 44
 Fränkel's instrument for collecting
 soil, 122
 pneumococcus, 150
 Friedländer's pneumobacillus, 150
 Fuchsin, anilin, 171
 basic, 18, 170
 Fuller and Johnson's groups of
 water bacteria, 112
 Fuller's method of titration, 52
 Fungi, 40

 GABBET'S blue, 172
 method for sputum, 154
 Gas, 77
 formula, 78
 production, 77
 tests for, 77
 Gelatin culture-medium, 51
 cultures, 61
 Gentian-violet, 18, 170
 anilin, 171
 Germicide, 44
 Germination of spores, 38
 Glanders, 133
 diagnosis of, 134
 inoculation of, 133
 isolation of bacterium, 135
 Gonococcus, 129, 130, 179
 Gonorrheal pus, examination of,
 129
 Graduated fermentation-tube, 57
 Gram's iodine solution, 19, 172
 method, 19
 stain, 19
 Granules, sulphur, 39, 41
 Green algæ, 40
 Grouping, study of, 85
 Groups, classification of bacteria by,
 107
 of milk bacteria, 112, 121
 of water bacteria, 112

 HALIBACTERIUM pellucidum, 175
 Hanging drop, 15
 Hematozoon malarix, 159
 Hesse's apparatus for collecting bac-
 teria in air, 120

 Hill fermentation-tube, 57
 Horsetails, 40
 Hot-air sterilization, 47
 sterilizer, 50
 Hunt's stain for diphtheria, 139
 Hyphomycetes, 40

 IDENTIFICATION of species, 83
 of yeasts and moulds, 168
 Immersion lens, 11
 oil, 11
 Immunity, production of, 85
 Impression preparation of colonies,
 75
 Indol production, 94
 Influenza, 141
 diagnosis of, 141
 Inoculation of anthrax, 130
 of diphtheria, 140
 of glanders, 133
 of plates, 66
 of pneumonia, 151
 of pus, 125
 of sputum, 151
 of tuberculosis, 155
 of tubes, 61
 Inoculations, 129
 intraperitoneal, 133
 intravenous, 125, 126, 151
 subcutaneous, 130, 140, 151, 155
 Instruments, sterilization of, 127
 Intraperitoneal inoculations, 133
 Intravenous inoculations, 125, 126
 151
 Involution forms, 90
 Iodine solution, Gram's, 19, 172
 Isolation of *Bacillus coli*, 119, 120
 typhosus, 115
 of *Bacterium diphtherix*, 137
 influenzæ, 141
 mallei, 135
 tuberculosis, 155
 of species, 81

 JEFFER'S counting plate, 117, 118
 Johne's method for capsules, 32

 KALTEYER'S cover-glass forceps,
 17
 Kitasato's filter, 46
 Koch-Ehrlich method for sections,
 156
 for sputum, 154
 Koch's syringe, 126
 Kühne's methylene-blue, 171

- LACTOSE, 56
 Lamprocystaceæ, 42
 Lamprocystis, 42
 Leprosy, 152
 Leptothrix buccalis, 176
 Lichenes, 40
 Lichens, 40
 Liebig's extract of beef, 55
 Life-history of a species, 83
 Liquefying ferment, 80
 Litmus milk, 58
 Liverworts, 40
 Löffler's blood-serum, 58
 method for flagella, 27
 modification of, 29
 methylene-blue, 171
 Lophotrichous, 25
- MALARIA, 159
 Malarial hematozoon, 159
 Measurement of bacteria, 11, 163
 Meat-extracts, 55
 Meat-sugar, 56
 Media, culture-, 51
 Merismopedia, 22
 flava varians, 180
 fragilis, 179
 goodsiri, 181
 mesenterica corrugata, 179
 mollis, 179
 ventriculi, 181
 Methylene-blue, 18, 170
 Kühne's, 171
 Löffler's, 171
 Unna's polychrome, 172
 Mica, 69
 Micrococci, form of, 22
 reproduction of, 34
 species of, 96
 Micrococcus, 39, 96
 albicans amplus, 178
 amylovorus, 175
 aureus, 124, 125
 cerasinus siccus, 181
 citreus, 125, 181
 conglomeratus, 179
 cumulatus tenuis, 180
 endocarditidis rugatus, 179
 flavus conjunctivæ, 179
 desidens, 179
 tardigradus, 179
 gonorrhœæ, 129
 lacteus faviformis, 179
 lucens, 179
 pflügeri, 179
 Micrococcus prodigiosus, 176
 pyæmiæ cuniculorum, 179
 pyogenes, 125, 178
 albus, 179
 aureus, 178
 tenuis, 180
 tetragenus, 125
 subflavus, 179
 ureæ liquefaciens, 179
 Micrometer, eyepiece, 12
 stage, 12
 Micromillimeter, 12
 Micron, 12
 Microscope, 11
 simple, for counting colonies, 116
 Microspira, 41, 106, 111
 comma, 27, 64, 180
 finkleri, 65
 metschnikovi, 65
 Migula, 39, 95
 Milk, analysis, 120
 classification of bacteria in, 112,
 121
 culture-medium, 58
 litmus, 58
 tubercle bacteria in, 157
 Mirror, 11
 Monas prodigiosa, 176
 Monotrichous, 25
 Mordant, 25, 27, 29
 Morphologic characters of a species.
 83, 84
 Morphology of a species, 83
 of bacteria, 22
 Mosses, 40
 Motility, test for, 90
 Motion, 24, 90
 Moulds, 165
 cultures of, 166
 examination of, 166
 identification of, 168
 staining, 166
 Mouse-holder, 129
 Movement, Brownian, 24, 25
 Mucor racemosus, 165, 166
 Museum preparations, 76
 Mycoderma aceti, 176
 Myconostoc gregarium, 181
 Mycoprotein, 19
 Mykobacterium tuberculosis, 178
 avium, 178
 Myxomycetes, 40
- NAME, determination of, 95
 Needle, platinum, 14

- Neisser's stain for diphtheria, 139
 Nessler's solution, 174
 Neutralization, 52
 Nitrates, 92
 test for, 93
 Nitrites, production of, 92
 test for, 92
 Nitrosomonas europæa, 181
 javanensis, 181
 Normal salt solution, 173
 Novy's jars, 79

 OBJECTIVES, 11
 Obligatory aërobes, 78
 anaërobes, 78

 PARAFFIN bath, 68
 Park's method for anaërobes, 80
 Pasteurization, 44
 Pathogenesis, 85, 94
 Pathogenic bacteria, 124
 Penicillium crustaceum, 167, 168
 Peridineæ, 40
 Peritrichous, 25
 Petri dish, 67
 Petri's sand filter, 121
 Phæophyceæ, 40
 Phanerogamia, 40
 Phenolphthalein, 52
 Phenol-sulphonic acid, 174
 Photobacterium annulare, 180
 caraibicum, 180
 coronatum, 180
 degenerans, 180
 delgadense, 180
 fischeri, 175
 glutinosum, 180
 indicum, 175
 javanense, 181
 luminosum, 180
 papillare, 180
 phosphorescens, 177, 179
 tuberosum, 180
 Photography, measuring bacteria
 by, 163
 Phragmidiothrix, 41
 Pigment, production of, 85
 Pipet, Chester and Robin, for ma-
 larial blood, 147, 148
 Pitfield's method for flagella, 25
 modification of, 26
 Planococcus, 39
 Planosarcina, 39
 Plants, 40
 Plate, counting, Jeffer's, 117, 118
 Plate, counting, Wolfhügel's, 116,
 117
 cultures, 66
 Platinum needles, 14
 wire, 14
 Pleomorphism, 90
 Pneumobacillus, 150
 liquefaciens bovis, 176
 Pneumococcus, 150
 Pneumonia, 150
 inoculation of, 151
 Polar staining, 24
 Polychrome methylene-blue, Unna's,
 172
 Porcelain filter, 45
 Potato, 55
 cultures, 66
 Potato-tubes, 56
 Production of acid, 94
 of alkali, 94
 of ammonia, 92
 of gas, 77
 of immunity, 85
 of indol, 94
 of liquefying ferment, 80
 of nitrites, 92
 of pigment, 85
 of spores, 35, 90
 of toxin, 85
 Proteus capsulatus septicus, 177
 mirabilis, 175,
 sulphureus, 176
 vulgaris, 176
 zenkeri, 176
 Pseudomonas, 39, 105
 Pteridophyta, 40
 Pure cultures, 60
 Pus, gonorrheal, staining of, 129
 inoculation of, 125
 Pyogenic organisms, 125

 QUALITATIVE analysis of water,
 115, 118
 Quantitative analysis of water, 114
 Quartan fever, 160

 REAGENTS, 170
 Red algæ, 40
 Reproduction by division, 34
 by spores, 35
 of bacteria, 34
 Rhabdochromatium, 42
 Rhodobacteriaceæ, 41
 Rhodophyceæ, 40

- Rosenberger's method for sections,
156
for sputum, 154
- SACCHAROMYCES cerevisiæ, 165,
166
- Saccharomycetaceæ, 168
- Saccharose, 56
- Safranin, 20, 170
- Salt solution, normal, 173
- Sand filter, Petri's, 121
- Sarcina, 34, 39, 98
fuscescens, 181
tetragera, 125, 128
- Schizomycetes, 39, 40
- Schizophyceæ, 40
- Schizophyta, 40
- Sections, staining, 20, 129
of actinomycosis, 158
of glanders, 133
of tuberculosis, 156
- Sedgwick's tube for air analysis,
121
- Sewage, 115
bacteria, 115
contamination, detection of, 115
- Slide for diphtheria, diagnosis, 140
for Widal reaction, 148, 149
- Slime-fungi, 40
- Sliver, 48
- Smegma bacterium, 152, 156, 178
- Smith fermentation-tube, 57
- Soil analysis, 122
bacteria, 122
- Species, determination of, 83
isolation of, 81
life-history of, 83
morphology of, 83
names of, 95
- Sphærococcus acidi lactici, 179
- Sphærotilus, 41
- Spirilla, 22
- Spirillaceæ, 41, 106
- Spirillum, 41
attenuatum, 181
cholerae Asiaticæ, 180
finkleri, 180
marinum, 180
- Spirochæta, 23, 41
- Spirosoma, 41, 106
- Spores, 34
endogenous, 34
germination of, 38
production of, 35, 90
reproduction by, 35
- Spores, staining, 36
test for, 90
- Sputum, examination of influenza,
43
of tuberculous, 152
inoculation of, 151
tubercle bacteria in, 153, 155, 157
- Stab cultures, 61
- Stain bottles, 18
Gram's, 19
Hunt's, 139
Neisser's, 139
- Staining actinomycosis, 158
anthrax blood, 131
bacteria in tissues, 20, 129
blood for malarial organism, 159,
161
preparations, 131, 161
diphtheria bacteria, 137, 138
flagella, 25
gonorrheal pus, 129
influenza sputum, 143
malarial blood, 159
pneumonia bacteria, 150
sections, 20, 129
for actinomycosis, 158
for glanders, 133
for tubercle bacteria, 156
spores, 36
tubercle bacteria, 152
- Stains, 18, 170
anilin, 170
ordinary, 16
- Staphylococcus, 22, 34
cereus albus, 178
aureus, 178
flavus, 178
pyogenes albus, 125, 179
aureus, 125, 178
citreus, 125, 178
salivarius pyogenes, 179
- Steam sterilization, 46
under pressure, 46
sterilizers, 46, 47, 48, 49
- Sterilization, 43
by hot air, 47
by steam, 46
under pressure, 46
discontinuous, 43
intermittent, 43
of instruments, 127
of syringes, 125
- Sterilizer, 46
Arnold, 46, 47, 48
hot-air, 50

- Sterilizer, steam, 46, 47, 48, 49
 Sternberg, 95
 Stewart's cover-glass forceps, 17
 Stonewort, 40
 Streptobacillus pseudotuberculosis
 rodentium, 178
 Streptococci, 22, 34
 species of, 95
 Streptococcus, 39, 95
 agalactiæ contagiosæ, 182
 brevis, 182
 coli gracilis, 182
 conglomeratus, 182
 coryzæ contagiosæ equorum, 182
 erysipelatos, 182
 giganteus urethræ, 182
 intracellularis, 179
 longus, 182
 mastitidis sporadicæ, 182
 murisepticus, 182
 pyogenes, 125, 127, 128
 septicus liquefaciens, 182
 liquefians, 182
 septo-pyæmicus, 182
 Stroke cultures, 64
 Subcutaneous inoculations, 130, 140,
 151, 155
 Synonyms, table of, 175
 Syphilis, 152
 Syringe, Koch's, 126
 Syringes, sterilization of, 125

 TERTIAN fever, 160
 Test for ammonia, 93
 for Bacillus coli, 118
 for motility, 90
 for nitrates, 93
 for nitrites, 92
 for spores, 90
 for virulence, Bact. diphtheriæ,
 139
 Tetragenococci, 22
 Thallophyta, 40
 Thermal death-point, determination
 of, 91
 Thiobacteria, 41
 Thiocapsa, 42
 Thiocapsacæ, 42
 Thiocystitis, 42
 Thiodictyon, 42
 Thionin blue, 170
 Thiopedia, 42
 Thiopediaceæ, 42
 Thiopolycoccus, 42
 Thiosarcina, 42
 Thiospirillum, 42
 Thiothece, 42
 Thiothrix, 41
 Tissues, staining bacteria in, 20,
 129
 in actinomycosis, 158
 in glanders, 133
 in tuberculosis, 156
 Titration, 52
 Toxin, production of, 85
 Triacid stain, Ehrlich's, 172
 Tubercle bacteria, cultures of,
 152
 in milk, 157
 in sputum, 152, 153, 155, 157
 in urine, 156
 staining, Gabbet's method, 154
 in sections, 156
 Koch-Ehrlich method, 154
 Rosenberger's method, 154
 Ziehl-Neelsen method, 152
 Tuberculosis, 151
 inoculation, 155
 Tuberculous sputum, examination
 of, 152
 Tubes, etched, 55
 Typhoid bacilli, 115, 144
 fever, 115, 144

 ULVINA aceti, 176
 Unna's polychrome methylene-blue,
 172
 Urine, tubercle bacteria in, 156

 VAN ERMENGEM'S method for fla-
 gella, 30
 modification of, 30
 Vaseline, 15
 Vibrio cholerae Asiaticæ, 180
 comma, 180
 dunbari, 180
 finkleri, 180
 flavescens, 181
 flavus, 181
 metschnikovi, 180
 proteus, 180
 saprophiles β , 180
 γ , 180
 tonsillaris, 180
 Virulence, test for, Bact. diphtheriæ,
 139

 WATER analysis, qualitative, 115
 quantitative, 114
 bacteria, classification of, 112

- Water bath, 68
 collection of, for analysis, 115
 contamination of, by sewage, 115
Welch's method for capsules, 31
White acid, 55
Whitney's method for blood, 161
Widal reaction, 145, 149
 slide, 148, 149
Wilson and Randolph's method for
 measuring bacteria, 163
Wire, platinum, 14
Wolfhügel's counting plate, 116,
 117
Wright's method for growing anaë-
 robes, 79
YEASTS, 165, 166
 cultures of, 166
 identification of, 168
 staining, 166
ZENKER'S fluid, 20, 173
Ziehl-Neelsen method for sections,
 156
 for sputum, 152
Ziehl's carbol-fuchsin, 172
Zylonite, 15

Catalogue ^{of} the Medical Publications

OF

W. B. SAUNDERS & COMPANY

PHILADELPHIA ✕ ✕ ✕ ✕ ✕ LONDON
925 Walnut Street ✕ ✕ ✕ ✕ ✕ 161 Strand, W. C.

Arranged Alphabetically and Classified under Subjects
See page 18 for a List of Contents classified according to subjects

THE books advertised in this Catalogue as being *sold by subscription* are usually to be obtained from travelling solicitors, but they will be sent direct from the office of publication (charges of shipment prepaid) upon receipt of the prices given. All the other books advertised are commonly for sale by booksellers in all parts of the United States; but books will be sent to any address, carriage prepaid, on receipt of the published price.

Money may be sent at the risk of the publisher in either of the following ways: A postal money order, an express money order, a bank check, and in a registered letter. Money sent in any other way is at the risk of the sender.

SPECIAL OFFER To physicians of approved credit books will be sent, post-paid, on the following terms: \$5.00 cash upon delivery of books, and monthly payments of \$5.00 thereafter until full amount is paid. Any one or two volumes will be sent on thirty days' time to those who do not care to make a larger purchase.

AN AMERICAN TEXT-BOOK OF APPLIED THERAPEUTICS.

Edited by JAMES C. WILSON, M. D., Professor of Practice of Medicine and of Clinical Medicine, Jefferson Medical College, Philadelphia. Handsome imperial octavo volume of 1326 pages. Illustrated. Cloth, \$7.00 net; Sheep or Half Morocco, \$8.00 net. *Sold by Subscription.*

AN AMERICAN TEXT-BOOK OF THE DISEASES OF CHILDREN. Second Edition, Revised.

Edited by LOUIS STARR, M. D., Consulting Pediatricist to the Maternity Hospital, etc.; assisted by THOMPSON S. WESTCOTT, M. D., Attending Physician to the Dispensary for Diseases of Children, Hospital of the University of Pennsylvania. Handsome imperial octavo volume of 1244 pages, profusely illustrated. Cloth, \$7.00 net; Sheep or Half Morocco, \$8.00 net. *Sold by Subscription.*

AN AMERICAN TEXT-BOOK OF DISEASES OF THE EYE, EAR, NOSE, AND THROAT.

Edited by G. E. DE SCHWEINITZ, M. D., Professor of Ophthalmology, Jefferson Medical College, Philadelphia; and B. ALEXANDER RANDALL, M. D., Clinical Professor of Diseases of the Ear, University of Pennsylvania. Imperial octavo, 1251 pages; 766 illustrations, 59 of them in colors. Cloth, \$7.00 net; Sheep or Half Morocco, \$8.00 net. *Sold by Subscription.*

AN AMERICAN TEXT-BOOK OF GENITO-URINARY AND SKIN DISEASES.

Edited by L. BOLTON BANGS, M. D., Professor of Genito-Urinary Surgery, University and Bellevue Hospital Medical College, New York ; and W. A. HARDAWAY, M. D., Professor of Diseases of the Skin, Missouri Medical College. Imperial octavo volume of 1229 pages, with 300 engravings and 20 full-page colored plates. Cloth, \$7.00 net ; Sheep or Half Morocco, \$8.00 net. *Sold by Subscription.*

AN AMERICAN TEXT-BOOK OF GYNECOLOGY, MEDICAL AND SURGICAL. Second Edition, Revised.

Edited by J. M. BALDY, M. D., Professor of Gynecology, Philadelphia Polyclinic, etc. Handsome imperial octavo volume of 718 pages ; 341 illustrations in the text, and 38 colored and half-tone plates. Cloth, \$6.00 net ; Sheep or Half Morocco, \$7.00 net. *Sold by Subscription.*

AN AMERICAN TEXT-BOOK OF LEGAL MEDICINE AND TOXICOLOGY.

Edited by FREDERICK PETERSON, M. D., Chief of Clinic, Nervous Department, College of Physicians and Surgeons, New York ; and WALTER S. HAINES, M. D., Professor of Chemistry, Pharmacy, and Toxicology, Rush Medical College, Chicago. *In Preparation.*

AN AMERICAN TEXT-BOOK OF OBSTETRICS.

Edited by RICHARD C. NORRIS, M. D. ; Art Editor, ROBERT L. DICKINSON, M. D. Handsome imperial octavo volume of 1014 pages ; nearly 900 beautiful colored and half-tone illustrations. Cloth, \$7.00 net ; Sheep or Half Morocco, \$8.00 net. *Sold by Subscription.*

AN AMERICAN TEXT-BOOK OF PATHOLOGY.

Edited by LUDWIG HEKTOEN, M. D., Professor of Pathology in Rush Medical College, Chicago ; and DAVID RIESMAN, M. D., Demonstrator of Pathologic Histology in the University of Pennsylvania. Handsome imperial octavo, over 1200 pages, profusely illustrated. *By Subscription.*

AN AMERICAN TEXT-BOOK OF PHYSIOLOGY. Second Edition, Revised, in Two Volumes.

Edited by WILLIAM H. HOWELL, PH. D., M. D., Professor of Physiology, Johns Hopkins University, Baltimore, Md. Two royal octavo volumes of about 600 pages each. Fully illustrated. Per volume : Cloth, \$3.00 net ; Sheep or Half Morocco, \$3.75 net.

AN AMERICAN TEXT-BOOK OF SURGERY. Third Edition.

Edited by WILLIAM W. KEEN, M. D., LL.D., F. R. C. S. (Hon.) ; and J. WILLIAM WHITE, M. D., PH. D. Handsome octavo volume of 1230 pages ; 496 wood-cuts and 37 colored and half-tone plates. Thoroughly revised and enlarged, with a section devoted to "The Use of the Röntgen Rays in Surgery." Cloth, \$7.00 net ; Sheep or Half Morocco, \$8.00 net.

THE NEW STANDARDTHE NEW STANDARD**THE AMERICAN ILLUSTRATED MEDICAL DICTIONARY.
Second Edition, Revised.**

For Practitioners and Students. A Complete Dictionary of the Terms used in Medicine, Surgery, Dentistry, Pharmacy, Chemistry, and the kindred branches, including much collateral information of an encyclopedic character, together with new and elaborate tables of Arteries, Muscles, Nerves, Veins, etc.; of Bacilli, Bacteria, Micrococci, Streptococci; Eponymic Tables of Diseases, Operations, Signs and Symptoms, Stains, Tests, Methods of Treatment, etc., etc. By W. A. NEWMAN DORLAND, A. M., M. D., Editor of the "American Pocket Medical Dictionary." Handsome large octavo, nearly 800 pages, bound in full flexible leather. Price, \$4.50 net; with thumb index, \$5.00 net.

Gives a Maximum Amount of Matter in a Minimum Space and at the Lowest Possible Cost.

This Revised Edition contains all the Latest Terms.

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

"I congratulate you upon giving to the profession a dictionary so compact in its structure, and so replete with information required by the busy practitioner and student. It is a necessity as well as an informed companion to every doctor. It should be upon the desk of every practitioner and student of medicine."—JOHN B. MURPHY, *Professor of Surgery and Clinical Surgery, Northwestern University Medical School, Chicago.*

THE AMERICAN POCKET MEDICAL DICTIONARY. Third Edition, Revised.

Edited by W. A. NEWMAN DORLAND, M. D., Assistant Obstetrician to the Hospital of the University of Pennsylvania; Fellow of the American Academy of Medicine. Containing the pronunciation and definition of the principal words used in medicine and kindred sciences, with 64 extensive tables. Handsomely bound in flexible leather, with gold edges. Price \$1.00 net; with thumb index, \$1.25 net.

THE AMERICAN YEAR-BOOK OF MEDICINE AND SURGERY.

A Yearly Digest of Scientific Progress and Authoritative Opinion in all branches of Medicine and Surgery, drawn from journals, monographs, and text-books of the leading American and Foreign authors and investigators. Arranged with editorial comments, by eminent American specialists, under the editorial charge of GEORGE M. GOULD, M. D. Year-Book of 1901 in two volumes—Vol. I. including *General Medicine*; Vol. II., *General Surgery*. Per volume: Cloth, \$3.00 net; Half Morocco, \$3.75 net. *Sold by Subscription.*

ABBOTT ON TRANSMISSIBLE DISEASES. Second Edition, Revised.

The Hygiene of Transmissible Diseases: their Causation, Modes of Dissemination, and Methods of Prevention. By A. C. ABBOTT, M. D., Professor of Hygiene and Bacteriology, University of Pennsylvania. Octavo, 351 pages, with numerous illustrations. Cloth, \$2.50 net.

ANDERS' PRACTICE OF MEDICINE. Fifth Revised Edition.

A Text-Book of the Practice of Medicine. By JAMES M. ANDERS, M. D., PH. D., LL. D., Professor of the Practice of Medicine and of Clinical Medicine, Medico-Chirurgical College, Philadelphia. Handsome octavo volume of 1292 pages, fully illustrated. Cloth, \$5.50 net; Sheep or Half Morocco, \$6.50 net.

BASTIN'S BOTANY.

Laboratory Exercises in Botany. By EDSON S. BASTIN, M. A., late Professor of Materia Medica and Botany, Philadelphia College of Pharmacy. Octavo, 536 pages, with 87 plates. Cloth, \$2.00 net.

BECK ON FRACTURES.

Fractures. By CARL BECK, M. D., Surgeon to St. Mark's Hospital and the New York German Poliklinik, etc. With an appendix on the Practical Use of the Röntgen Rays. 335 pages, 170 illustrations. Cloth, \$3.50 net.

BECK'S SURGICAL ASEPSIS.

A Manual of Surgical Asepsis. By CARL BECK, M. D., Surgeon to St. Mark's Hospital and the New York German Poliklinik, etc. 306 pages; 65 text-illustrations and 12 full-page plates. Cloth, \$1.25 net.

BOISLINIÈRE'S OBSTETRIC ACCIDENTS, EMERGENCIES, AND OPERATIONS.

Obstetric Accidents, Emergencies, and Operations. By L. CH. BOISLINIÈRE, M. D., late Emeritus Professor of Obstetrics, St. Louis Medical College. 381 pages, handsomely illustrated. Cloth, \$2.00 net.

BÖHM, DAVIDOFF, AND HUBER'S HISTOLOGY.

A Text-Book of Human Histology. Including Microscopic Technic. By DR. A. A. BOHM and DR. M. VON DAVIDOFF, of Munich, and G. CARL HUBER, M. D., Junior Professor of Anatomy and Director of Histological Laboratory, University of Michigan. Handsome octavo of 501 pages, with 351 beautiful original illustrations. Cloth, \$3.50 net.

BUTLER'S MATERIA MEDICA, THERAPEUTICS, AND PHARMACOLOGY. Third Edition, Revised.

A Text-Book of Materia Medica, Therapeutics, and Pharmacology. By GEORGE F. BUTLER, PH. G., M. D., Professor of Materia Medica and of Clinical Medicine, College of Physicians and Surgeons, Chicago. Octavo, 874 pages, illustrated. Cloth, \$4.00 net; Sheep or Half Morocco, \$5.00 net.

CERNA ON THE NEWER REMEDIES. Second Edition, Revised.

Notes on the Newer Remedies, their Therapeutic Applications and Modes of Administration. By DAVID CERNA, M. D., PH. D., Demonstrator of Physiology, Medical Department, University of Texas. Rewritten and greatly enlarged. Post-octavo, 253 pages. Cloth, \$1.00 net.

CHAPIN ON INSANITY.

A Compendium of Insanity. By JOHN B. CHAPIN, M. D., LL.D., Physician-in-Chief, Pennsylvania Hospital for the Insane; Honorary Member of the Medico-Psychological Society of Great Britain, of the Society of Mental Medicine of Belgium, etc. 12mo, 234 pages, illustrated. Cloth, \$1.25 net.

**CHAPMAN'S MEDICAL JURISPRUDENCE AND TOXICOLOGY.
Second Edition, Revised.**

Medical Jurisprudence and Toxicology. By HENRY C. CHAPMAN, M. D., Professor of Institutes of Medicine and Medical Jurisprudence, Jefferson Medical College of Philadelphia. 254 pages, with 55 illustrations and 3 full-page plates in colors. Cloth, \$1.50 net.

CHURCH AND PETERSON'S NERVOUS AND MENTAL DISEASES. Third Edition, Revised and Enlarged.

Nervous and Mental Diseases. By ARCHIBALD CHURCH, M. D., Professor of Nervous and Mental Diseases, and Head of the Neurological Department, Northwestern University Medical School, Chicago; and FREDERICK PETERSON, M. D., Chief of Clinic, Nervous Department, College of Physicians and Surgeons, New York. Handsome octavo volume of 875 pages, profusely illustrated. Cloth, \$5.00 net; Sheep or Half Morocco, \$6.00 net.

CLARKSON'S HISTOLOGY.

A Text-Book of Histology, Descriptive and Practical. By ARTHUR CLARKSON, M. B., C. M. Edin., formerly Demonstrator of Physiology in the Owen's College, Manchester; late Demonstrator of Physiology in Yorkshire College, Leeds. Large octavo, 554 pages; 22 engravings and 174 beautifully colored original illustrations. Cloth, \$4.00 net.

CORWIN'S PHYSICAL DIAGNOSIS. Third Edition, Revised.

Essentials of Physical Diagnosis of the Thorax. By ARTHUR M. CORWIN, A. M., M. D., Instructor in Physical Diagnosis in Rush Medical College, Chicago. 219 pages, illustrated. Cloth, \$1.25 net.

CROOKSHANK'S BACTERIOLOGY. Fourth Edition, Revised.

A Text-Book of Bacteriology. By EDGAR M. CROOKSHANK, M. B., Professor of Comparative Pathology and Bacteriology, King's College, London. Octavo, 700 pages, 273 engravings and 22 original colored plates. Cloth, \$6.50 net; Half Morocco, \$7.50 net.

DACOSTA'S SURGERY. Third Edition, Revised.

Modern Surgery, General and Operative. By JOHN CHALMERS DACOSTA, M. D., Professor of Principles of Surgery and Clinical Surgery, Jefferson Medical College, Philadelphia; Surgeon to the Philadelphia Hospital, etc. Handsome octavo volume of 1117 pages, profusely illustrated. Cloth, \$5.00 net; Sheep or Half Morocco, \$6.00 net.

Enlarged by over 200 Pages, with more than 100 New Illustrations.

DAVIS'S OBSTETRIC NURSING.

Obstetric and Gynecologic Nursing. By EDWARD P. DAVIS, A. M., M. D., Professor of Obstetrics in Jefferson Medical College and the Philadelphia Polyclinic; Obstetrician and Gynecologist to the Philadelphia Hospital. 12mo volume of 400 pages, fully illustrated. Crushed buckram, \$1.75 net.

DE SCHWEINITZ ON DISEASES OF THE EYE. Third Edition, Revised.

Diseases of the Eye. A Handbook of Ophthalmic Practice. By G. E. DE SCHWEINITZ, M. D., Professor of Ophthalmology, Jefferson Medical College, Philadelphia, etc. Handsome royal octavo volume of 696 pages; 256 fine illustrations and 2 chromo-lithographic plates. Cloth, \$4.00 net; Sheep or Half Morocco, \$5.00 net.

DORLAND'S DICTIONARIES.

[See *American Illustrated Medical Dictionary* and *American Pocket Medical Dictionary* on page 3.]

DORLAND'S OBSTETRICS. Second Edition, Revised and Greatly Enlarged.

Modern Obstetrics. By W. A. NEWMAN DORLAND, M. D., Assistant Demonstrator of Obstetrics, University of Pennsylvania; Associate in Gynecology, Philadelphia Polyclinic. Octavo volume of 797 pages, with 201 illustrations. Cloth, \$4.00 net.

EICHHORST'S PRACTICE OF MEDICINE.

A Text-Book of the Practice of Medicine. By DR. HERMAN EICHHORST, Professor of Special Pathology and Therapeutics and Director of the Medical Clinic, University of Zurich. Translated and edited by AUGUSTUS A. ESHNER, M. D., Professor of Clinical Medicine, Philadelphia Polyclinic. Two royal octavo volumes, 600 pages each, 150 illustrations. Per set: Cloth, \$6.00 net; Sheep or Half Morocco, \$7.50 net.

FRIEDRICH AND CURTIS ON THE NOSE, THROAT, AND EAR.

Rhinology, Laryngology, and Otology, and their Significance in General Medicine. By DR. E. P. FRIEDRICH, of Leipzig. Edited by H. HOLBROOK CURTIS, M. D., Consulting Surgeon to the New York Nose and Throat Hospital. Octavo, 348 pages. Cloth, \$2.50 net.

FROTHINGHAM'S GUIDE FOR THE BACTERIOLOGIST.

Laboratory Guide for the Bacteriologist. By LANGDON FROTHINGHAM, M. D. V., Assistant in Bacteriology and Veterinary Science, Sheffield Scientific School, Yale University. Illustrated. Cloth, 75 cts. net.

GARRIGUES' DISEASES OF WOMEN. Third Edition, Revised.

Diseases of Women. By HENRY J. GARRIGUES, A. M., M. D., Gynecologist to St. Mark's Hospital and to the German Dispensary, New York City. Octavo, 756 pages, with 367 engravings and colored plates. Cloth, \$4.50 net; Sheep or Half Morocco, \$5.50 net.

GOULD AND PYLE'S CURIOSITIES OF MEDICINE.

Anomalies and Curiosities of Medicine. By GEORGE M. GOULD, M. D., and WALTER L. PYLE, M. D. An encyclopedic collection of rare and extraordinary cases and of the most striking instances of abnormality in all branches of Medicine and Surgery, derived from an exhaustive research of medical literature from its origin to the present day, abstracted, classified, annotated, and indexed. Handsome octavo volume of 968 pages; 295 engravings and 12 full-page plates. Popular Edition. Cloth, \$3.00 net; Sheep or Half Morocco, \$4.00 net.

GRAFSTROM'S MECHANO-THERAPY.

A Text-Book of Mechano-Therapy (Massage and Medical Gymnastics). By AXEL V. GRAFSTROM, B. SC., M. D., late House Physician, City Hospital, Blackwell's Island, New York. 12mo, 139 pages, illustrated. Cloth, \$1.00 net.

GRIFFITH ON THE BABY. Second Edition, Revised.

The Care of the Baby. By J. P. CROZER GRIFFITH, M. D., Clinical Professor of Diseases of Children, University of Pennsylvania; Physician to the Children's Hospital, Philadelphia, etc. 12mo, 404 pages, 67 illustrations and 5 plates. Cloth, \$1.50 net.

GRIFFITH'S WEIGHT CHART.

Infant's Weight Chart. Designed by J. P. CROZER GRIFFITH, M. D., Clinical Professor of Diseases of Children, University of Pennsylvania. 25 charts in each pad. Per pad, 50 cts. net.

HART'S DIET IN SICKNESS AND IN HEALTH.

Diet in Sickness and Health. By MRS. ERNEST HART, formerly Student of the Faculty of Medicine of Paris and of the London School of Medicine for Women; with an Introduction by SIR HENRY THOMPSON, F. R. C. S., M. D., London. 220 pages. Cloth, \$1.50 net.

HAYNES' ANATOMY.

A Manual of Anatomy. By IRVING S. HAYNES, M. D., Professor of Practical Anatomy in Cornell University Medical College. 680 pages; 42 diagrams and 134 full-page half-tone illustrations from original photographs of the author's dissections. Cloth, \$2.50 net.

HEISLER'S EMBRYOLOGY. Second Edition, Revised.

A Text-Book of Embryology. By JOHN C. HEISLER, M. D., Professor of Anatomy, Medico-Chirurgical College, Philadelphia. Octavo volume of 405 pages, handsomely illustrated. Cloth, \$2.50 net.

HIRST'S OBSTETRICS. Third Edition, Revised and Enlarged.

A Text-Book of Obstetrics. By BARTON COOKE HIRST, M. D., Professor of Obstetrics, University of Pennsylvania. Handsome octavo volume of 873 pages, 704 illustrations, 36 of them in colors. Cloth, \$5.00 net; Sheep or Half Morocco, \$6.00 net.

HYDE & MONTGOMERY ON SYPHILIS AND THE VENEREAL DISEASES. 2d Edition, Revised and Greatly Enlarged.

Syphilis and the Venereal Diseases. By JAMES NEVINS HYDE, M. D., Professor of Skin and Venereal Diseases, and FRANK H. MONTGOMERY, M. D., Associate Professor of Skin, Genito-Urinary, and Venereal Diseases in Rush Medical College, Chicago, Ill. Octavo, 594 pages, profusely illustrated. Cloth, \$4.00 net.

THE INTERNATIONAL TEXT-BOOK OF SURGERY. In Two Volumes.

By American and British Authors. Edited by J. COLLINS WARREN, M. D., LL. D., F. R. C. S. (Hon.), Professor of Surgery, Harvard Medical School, Boston; and A. PEARCE GOULD, M. S., F. R. C. S., Lecturer on Practical Surgery and Teacher of Operative Surgery, Middlesex Hospital Medical School, London, Eng. Vol. I. *General Surgery*.—Handsome octavo, 947 pages, with 458 beautiful illustrations and 9 lithographic plates. Vol. II. *Special or Regional Surgery*.—Handsome octavo, 1072 pages, with 471 beautiful illustrations and 8 lithographic plates. *Sold by Subscription*. Prices per volume: Cloth, \$5.00 net; Sheep or Half Morocco, \$6.00 net.

"It is the most valuable work on the subject that has appeared in some years. The clinician and the pathologist have joined hands in its production, and the result must be a satisfaction to the editors as it is a gratification to the conscientious reader."—*Annals of Surgery*.

"This is a work which comes to us on its own intrinsic merits. Of the latter it has very many. The arrangement of subjects is excellent, and their treatment by the different authors is equally so. What is especially to be recommended is the painstaking endeavor of each writer to make his subject clear and to the point. To this end particularly is the technique of operations lucidly described in all necessary detail. And withal the work is up to date in a very remarkable degree, many of the latest operations in the different regional parts of the body being given in full details. There is not a chapter in the work from which the reader may not learn something new."—*Medical Record*, New York.

JACKSON'S DISEASES OF THE EYE.

A Manual of Diseases of the Eye. By EDWARD JACKSON, A. M., M. D., Emeritus Professor of Diseases of the Eye, Philadelphia Polyclinic and College for Graduates in Medicine. 12mo, volume of 535 pages, with 178 illustrations, mostly from drawings by the author. Cloth, \$2.50 net.

KEATING'S LIFE INSURANCE.

How to Examine for Life Insurance. By JOHN M. KEATING, M. D., Fellow of the College of Physicians of Philadelphia; Ex-President of the Association of Life Insurance Medical Directors. Royal octavo, 211 pages. With numerous illustrations. Cloth, \$2.00 net.

KEEN ON THE SURGERY OF TYPHOID FEVER.

The Surgical Complications and Sequels of Typhoid Fever. By WM. W. KEEN, M. D., LL.D., F. R. C. S. (Hon.), Professor of the Principles of Surgery and of Clinical Surgery, Jefferson Medical College, Philadelphia, etc. Octavo volume of 386 pages, illustrated. Cloth, \$3.00 net.

KEEN'S OPERATION BLANK. Second Edition, Revised Form.

An Operation Blank, with Lists of Instruments, etc. Required in Various Operations. Prepared by W. W. KEEN, M. D., LL.D., F. R. C. S. (Hon.), Professor of the Principles of Surgery and of Clinical Surgery, Jefferson Medical College, Philadelphia. Price per pad, of 50 blanks, 50 cts. net.

KYLE ON THE NOSE AND THROAT. Second Edition.

Diseases of the Nose and Throat. By D. BRADEN KYLE, M. D., Clinical Professor of Laryngology and Rhinology, Jefferson Medical College, Philadelphia. Octavo, 646 pages; over 150 illustrations and 6 lithographic plates. Cloth, \$4.00 net; Sheep or Half Morocco, \$5.00 net.

LAINÉ'S TEMPERATURE CHART.

Temperature Chart. Prepared by D. T. LAINÉ, M. D. Size 8 x 13½ inches. A conveniently arranged Chart for recording Temperature, with columns for daily amounts of Urinary and Fecal Excretions, Food, Remarks, etc. On the back of each chart is given the Brand treatment of Typhoid Fever. Price, per pad of 25 charts, 50 cts. net.

LEVY, KLEMPERER, AND ESHNER'S CLINICAL BACTERIOLOGY.

The Elements of Clinical Bacteriology. By DR. ERNST LEVY, Professor in the University of Strasburg, and DR. FELIX KLEMPERER, Privatdocent in the University of Strasburg. Translated and edited by AUGUSTUS A. ESHNER, M. D., Professor of Clinical Medicine, Philadelphia Polyclinic. Octavo, 440 pages, fully illustrated. Cloth, \$2.50 net.

LOCKWOOD'S PRACTICE OF MEDICINE. Second Edition, Revised and Enlarged.

A Manual of the Practice of Medicine. By GEORGE ROE LOCKWOOD, M. D., Professor of Practice in the Women's Medical College of the New York Infirmary, etc.

LONG'S SYLLABUS OF GYNECOLOGY.

A Syllabus of Gynecology, arranged in Conformity with "An American Text-Book of Gynecology." By J. W. LONG, M. D., Professor of Diseases of Women and Children, Medical College of Virginia, etc. Cloth, interleaved, \$1.00 net.

MACDONALD'S SURGICAL DIAGNOSIS AND TREATMENT.

Surgical Diagnosis and Treatment. By J. W. MACDONALD, M. D. Edin., F. R. C. S. Edin., Professor of Practice of Surgery and Clinical Surgery, Hamline University. Handsome octavo, 800 pages, fully illustrated. Cloth, \$5.00 net; Sheep or Half Morocco, \$6.00 net.

MALLORY AND WRIGHT'S PATHOLOGICAL TECHNIQUE. Second Edition, Revised and Enlarged.

Pathological Technique. A Practical Manual for Laboratory Work in Pathology, Bacteriology, and Morbid Anatomy, with chapters on Post-Mortem Technique and the Performance of Autopsies. By FRANK B. MALLORY, A. M., M. D., Assistant Professor of Pathology, Harvard University Medical School, Boston; and JAMES H. WRIGHT, A. M., M. D., Instructor in Pathology, Harvard University Medical School, Boston.

McFARLAND'S PATHOGENIC BACTERIA. Third Edition, increased in size by over 100 Pages.

Text-Book upon the Pathogenic Bacteria. By JOSEPH McFARLAND, M. D., Professor of Pathology and Bacteriology, Medico-Chirurgical College of Philadelphia, etc. Octavo volume of 621 pages, finely illustrated. Cloth, \$3.25 net.

MEIGS ON FEEDING IN INFANCY.

Feeding in Early Infancy. By ARTHUR V. MEIGS, M. D. Bound in limp cloth, flush edges, 25 cts. net.

MOORE'S ORTHOPEDIC SURGERY.

A Manual of Orthopedic Surgery. By JAMES E. MOORE, M. D., Professor of Orthopedics and Adjunct Professor of Clinical Surgery, University of Minnesota, College of Medicine and Surgery. Octavo volume of 356 pages, handsomely illustrated. Cloth, \$2.50 net.

MORTEN'S NURSES' DICTIONARY.

Nurses' Dictionary of Medical Terms and Nursing Treatment. Containing Definitions of the Principal Medical and Nursing Terms and Abbreviations; of the Instruments, Drugs, Diseases, Accidents, Treatments, Operations, Foods, Appliances, etc. encountered in the ward or in the sick-room. By HONOR MORTEN, author of "How to Become a Nurse," etc. 16mo, 140 pages. Cloth, \$1.00 net.

NANCREDE'S ANATOMY AND DISSECTION. Fourth Edition.

Essentials of Anatomy and Manual of Practical Dissection. By CHARLES B. NANCREDE, M. D., LL.D., Professor of Surgery and of Clinical Surgery, University of Michigan, Ann Arbor. Post-octavo, 500 pages, with full-page lithographic plates in colors and nearly 200 illustrations. Extra Cloth (or Oilcloth for dissection-room), \$2.00 net.

NANCREDE'S PRINCIPLES OF SURGERY.

Lectures on the Principles of Surgery. By CHARLES B. NANCREDE, M. D., LL.D., Professor of Surgery and of Clinical Surgery, University of Michigan, Ann Arbor. Octavo, 398 pages, illustrated. Cloth, \$2.50 net.

NORRIS'S SYLLABUS OF OBSTETRICS. Third Edition, Revised.

Syllabus of Obstetrical Lectures in the Medical Department of the University of Pennsylvania. By RICHARD C. NORRIS, A. M., M. D., Instructor in Obstetrics and Lecturer on Clinical and Operative Obstetrics, University of Pennsylvania. Crown octavo, 222 pages. Cloth, interleaved for notes, \$2.00 net.

OGDEN ON THE URINE.

Clinical Examination of the Urine and Urinary Diagnosis. A Clinical Guide for the Use of Practitioners and Students of Medicine and Surgery. By J. BERGEN OGDEN, M. D., Instructor in Chemistry, Harvard University Medical School. Handsome octavo, 416 pages, with 54 illustrations, and a number of colored plates. Cloth, \$3.00 net.

PENROSE'S DISEASES OF WOMEN. Fourth Edition, Revised.

A Text-Book of Diseases of Women. By CHARLES B. PENROSE, M. D., PH. D., formerly Professor of Gynecology in the University of Pennsylvania. Octavo volume of 538 pages, handsomely illustrated. Cloth, \$3.75 net.

PRYOR—PELVIC INFLAMMATIONS.

The Treatment of Pelvic Inflammations through the Vagina. By W. R. PRYOR, M. D., Professor of Gynecology, New York Polyclinic. 12mo, 248 pages, handsomely illustrated. Cloth, \$2.00 net.

PYE'S BANDAGING.

Elementary Bandaging and Surgical Dressing. With Directions concerning the Immediate Treatment of Cases of Emergency. By WALTER PYE, F. R. C. S., late Surgeon to St. Mary's Hospital, London. Small 12mo, over 80 illustrations. Cloth, flexible covers, 75 cts. net.

PYLE'S PERSONAL HYGIENE.

A Manual of Personal Hygiene. Proper Living upon a Physiologic Basis. Edited by WALTER L. PYLE, M. D., Assistant Surgeon to the Wills Eye Hospital, Philadelphia. Octavo volume of 344 pages, fully illustrated. Cloth, \$1.50 net.

RAYMOND'S PHYSIOLOGY. Second Edition, Revised and Greatly Enlarged.

A Text-Book of Physiology. By JOSEPH H. RAYMOND, A. M., M. D., Professor of Physiology and Hygiene and Lecturer on Gynecology in the Long Island College Hospital.

SALINGER AND KALTEYER'S MODERN MEDICINE.

Modern Medicine. By JULIUS L. SALINGER, M. D., Demonstrator of Clinical Medicine, Jefferson Medical College; and F. J. KALTEYER, M. D., Assistant Demonstrator of Clinical Medicine, Jefferson Medical College. Handsome octavo, 801 pages, illustrated. Cloth, \$4.00 net.

SAUNDBY'S RENAL AND URINARY DISEASES.

Lectures on Renal and Urinary Diseases. By ROBERT SAUNDBY, M. D. Edin., Fellow of the Royal College of Physicians, London, and of the Royal Medico-Chirurgical Society; Professor of Medicine in Mason College, Birmingham, etc. Octavo, 434 pages, with numerous illustrations and 4 colored plates. Cloth, \$2.50 net.

SAUNDERS' MEDICAL HAND-ATLASES. See pp. 16 and 17.**SAUNDERS' POCKET MEDICAL FORMULARY. Sixth Edition, Revised.**

By WILLIAM M. POWELL, M. D., author of "Essentials of Diseases of Children"; Member of Philadelphia Pathological Society. Containing 1844 formulæ from the best-known authorities. With an Appendix containing Posological Table, Formulæ and Doses for Hypodermic Medication, Poisons and their Antidotes, Diameters of the Female Pelvis and Fetal Head, Obstetrical Table, Diet List for Various Diseases, Materials and Drugs used in Antiseptic Surgery, Treatment of Asphyxia from Drowning, Surgical Remembrancer, Tables of Incompatibles, Eruptive Fevers, etc., etc. Handsomely bound in flexible morocco, with side index, wallet, and flap. \$2.00 net.

SAUNDERS' QUESTION-COMPENDS. See pages 14 and 15.

SCUDDER'S FRACTURES. Second Edition, Revised.

The Treatment of Fractures. By CHAS L. SCUDDER, M. D., Assistant in Clinical and Operative Surgery, Harvard University Medical School. Octavo, 433 pages, with nearly 600 original illustrations. Polished Buckram, \$4.50 net; Half Morocco, \$5.50 net.

SENN'S GENITO-URINARY TUBERCULOSIS.

Tuberculosis of the Genito-Urinary Organs, Male and Female. By NICHOLAS SENN, M. D., PH. D., LL.D., Professor of the Practice of Surgery and of Clinical Surgery, Rush Medical College, Chicago. Handsome octavo volume of 320 pages, illustrated. Cloth, \$3.00 net.

SENN'S PRACTICAL SURGERY.

Practical Surgery. By NICHOLAS SENN, M. D., PH. D., LL.D., Professor of the Practice of Surgery and of Clinical Surgery, Rush Medical College, Chicago. Handsome octavo volume of 1200 pages, profusely illustrated. Cloth, \$6.00 net; Sheep or Half Morocco, \$7.00 net. *By Subscription.*

SENN'S SYLLABUS OF SURGERY.

A Syllabus of Lectures on the Practice of Surgery, arranged in conformity with "An American Text-Book of Surgery." By NICHOLAS SENN, M. D., PH.D., LL.D., Professor of the Practice of Surgery and of Clinical Surgery, Rush Medical College, Chicago. Cloth, \$1.50 net.

SENN'S TUMORS. Second Edition, Revised.

Pathology and Surgical Treatment of Tumors. By NICHOLAS SENN, M. D., PH.D., LL.D., Professor of the Practice of Surgery and of Clinical Surgery, Rush Medical College, Chicago. Octavo volume of 718 pages, with 478 illustrations, including 12 full-page plates in colors. Cloth, \$5.00 net; Sheep or Half Morocco, \$6.00 net.

STARR'S DIETS FOR INFANTS AND CHILDREN.

Diets for Infants and Children in Health and in Disease. By LOUIS STARR, M. D., Editor of "An American Text-Book of the Diseases of Children." 230 blanks (pocket-book size), perforated and neatly bound in flexible morocco. \$1.25 net.

STENGEL'S PATHOLOGY. Third Edition, Thoroughly Revised.

A Text-Book of Pathology. By ALFRED STENGEL, M. D., Professor of Clinical Medicine, University of Pennsylvania; Visiting Physician to the Pennsylvania Hospital. Handsome octavo, 873 pages, nearly 400 illustrations, many of them in colors. Cloth, \$5.00 net; Sheep or Half Morocco, \$6.00 net.

STENGEL AND WHITE ON THE BLOOD.

The Blood in its Clinical and Pathological Relations. By ALFRED STENGEL, M. D., Professor of Clinical Medicine, University of Pennsylvania; and C. Y. WHITE, JR., M. D., Instructor in Clinical Medicine, University of Pennsylvania. *In Press.*

STEVENS' MATERIA MEDICA AND THERAPEUTICS. Third Edition, Revised and Greatly Enlarged.

A Text-Book of Modern Therapeutics. By A. A. STEVENS, A. M., M. D., Lecturer on Physical Diagnosis in the University of Pennsylvania.

STEVENS' PRACTICE OF MEDICINE. Fifth Edition, Revised.

A Manual of the Practice of Medicine. By A. A. STEVENS, A. M., M. D., Lecturer on Physical Diagnosis in the University of Pennsylvania. Specially intended for students preparing for graduation and hospital examinations. Post-octavo, 519 pages; illustrated. Flexible Leather, \$2.00 net.

STEWART'S PHYSIOLOGY. Fourth Edition, Revised.

A Manual of Physiology, with Practical Exercises. For Students and Practitioners. By G. N. STEWART, M. A., M. D., D. Sc., Professor of Physiology in the Western Reserve University, Cleveland, Ohio. Octavo volume of 894 pages; 336 illustrations and 5 colored plates. Cloth, \$3.75 net.

STONE'S MATERIA MEDICA FOR NURSES.

Materia Medica for Nurses. By EMILY A. M. STONEY, late Superintendent of the Training-School for Nurses, Carney Hospital, South Boston, Mass. Handsome octavo volume of 306 pages. Cloth, \$1.50 net.

STONE'S NURSING. Second Edition, Revised.

Practical Points in Nursing. For Nurses in Private Practice. By EMILY A. M. STONEY, late Superintendent of the Training-School for Nurses, Carney Hospital, South Boston, Mass. 456 pages, with 73 engravings and 8 colored and half-tone plates. Cloth, \$1.75 net.

STONE'S SURGICAL TECHNIC FOR NURSES.

Bacteriology and Surgical Technic for Nurses. By EMILY A. M. STONEY, late Superintendent of the Training-School for Nurses, Carney Hospital, South Boston, Mass. 12mo volume, fully illustrated. Cloth, \$1.25 net.

THOMAS'S DIET LISTS. Second Edition, Revised.

Diet Lists and Sick-Room Dietary. By JEROME B. THOMAS, M. D., Instructor in Materia Medica, Long Island Hospital; Assistant Bacteriologist to the Hoagland Laboratory. Cloth, \$1.25 net. Send for sample sheet.

THORNTON'S DOSE-BOOK AND PRESCRIPTION-WRITING. Second Edition, Revised and Enlarged.

Dose-Book and Manual of Prescription-Writing. By E. Q. THORNTON, M. D., Demonstrator of Therapeutics, Jefferson Medical College, Philadelphia.

VAN VALZAH AND NISBET'S DISEASES OF THE STOMACH.

Diseases of the Stomach. By WILLIAM W. VAN VALZAH, M. D., Professor of General Medicine and Diseases of the Digestive System and the Blood, New York Polyclinic; and J. DOUGLAS NISBET, M. D., Adjunct Professor of General Medicine and Diseases of the Digestive System and the Blood, New York Polyclinic. Octavo volume of 674 pages, illustrated. Cloth, \$3.50 net.

VECKI'S SEXUAL IMPOTENCE. Second Edition, Revised.

The Pathology and Treatment of Sexual Impotence. By VICTOR G. VECKI, M. D. From the second German edition, revised and enlarged. Demi-octavo, 291 pages. Cloth, \$2.00 net.

VIERORDT'S MEDICAL DIAGNOSIS. Fourth Edition, Revised.

Medical Diagnosis. By DR. OSWALD VIERORDT, Professor of Medicine, University of Heidelberg. Translated, with additions, from the fifth enlarged German edition, with the author's permission, by FRANCIS H. STUART, A. M., M. D. Handsome octavo volume, 603 pages; 194 woodcuts, many of them in colors. Cloth, 4.00 net; Sheep or Half-Morocco, \$5.00 net.

WATSON'S HANDBOOK FOR NURSES.

A Handbook for Nurses. By J. K. WATSON, M. D. Edin. American Edition, under supervision of A. A. STEVENS, A. M., M. D., Lecturer on Physical Diagnosis, University of Pennsylvania. 12mo, 413 pages, 73 illustrations. Cloth, \$1.50 net.

WARREN'S SURGICAL PATHOLOGY. Second Edition.

Surgical Pathology and Therapeutics. By JOHN COLLINS WARREN, M. D., LL.D., F. R. C. S. (Hon.), Professor of Surgery, Harvard Medical School. Handsome octavo, 873 pages; 136 relief and lithographic illustrations, 33 in colors. With an Appendix on Scientific Aids to Surgical Diagnosis, and a series of articles on Regional Bacteriology. Cloth, \$5.00 net; Sheep or Half Morocco, \$6.00 net.

SAUNDERS'

QUESTION=COMPENDS,

ARRANGED IN QUESTION AND ANSWER FORM.

The Most Complete and Best Illustrated Series of Compends Ever Issued.

NOW THE STANDARD AUTHORITIES IN MEDICAL LITERATURE

WITH

Students and Practitioners in every City of the United States and Canada.

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

OVER 200,000 COPIES

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

SEE NEXT PAGE FOR LIST.

Saunders' Question-Compend Series.

Price, Cloth, \$1.00 net per copy, except when otherwise noted.

"Where the work of preparing students' manuals is to end we cannot say, but the Saunders Series, in our opinion, bears off the palm at present."—*New York Medical Record*.

1. **Essentials of Physiology.** By SIDNEY BUDGETT, M. D. *An entirely new work.*
2. **Essentials of Surgery.** By EDWARD MARTIN, M. D. Seventh edition, revised, with an Appendix and a chapter on Appendicitis.
3. **Essentials of Anatomy.** By CHARLES B. NANCREDÉ, M. D. Sixth edition, thoroughly revised and enlarged.
4. **Essentials of Medical Chemistry, Organic and Inorganic.** By LAWRENCE WOLFF, M. D. Fifth edition, revised.
5. **Essentials of Obstetrics.** By W. EASTERLY ASHTON, M. D. Fourth edition, revised and enlarged.
6. **Essentials of Pathology and Morbid Anatomy.** By F. J. KALTEYER, M. D. *In preparation.*
7. **Essentials of Materia Medica, Therapeutics, and Prescription-Writing.** By HENRY MORRIS, M. D. Fifth edition, revised.
- 8, 9. **Essentials of Practice of Medicine.** By HENRY MORRIS, M. D. An Appendix on URINE EXAMINATION. By LAWRENCE WOLFF, M. D. Third edition, enlarged by some 300 Essential Formulæ, selected from eminent authorities, by WM. M. POWELL, M. D. (Double number, \$1.50 net.)
10. **Essentials of Gynecology.** By EDWIN B. CRAGIN, M. D. Fifth edition, revised.
11. **Essentials of Diseases of the Skin.** By HENRY W. STELWAGON, M. D. Fourth edition, revised and enlarged.
12. **Essentials of Minor Surgery, Bandaging, and Venereal Diseases.** By EDWARD MARTIN, M. D. Second edition, revised and enlarged.
13. **Essentials of Legal Medicine, Toxicology, and Hygiene.** This volume is at present out of print.
14. **Essentials of Diseases of the Eye.** By EDWARD JACKSON, M. D. Third edition, revised and enlarged.
15. **Essentials of Diseases of Children.** By WILLIAM M. POWELL, M. D. Third
16. **Essentials of Examination of Urine.** By LAWRENCE WOLFF, M. D. Colored "VOGEL SCALE." (75 cents net.)
17. **Essentials of Diagnosis.** By S. SOLIS-COHEN, M. D., and A. A. ESHNER, M. D. Second edition, thoroughly revised.
18. **Essentials of Practice of Pharmacy.** By LUCIUS E. SAYRE. Second edition, revised and enlarged.
19. **Essentials of Diseases of the Nose and Throat.** By E. B. GLEASON, M. D. Third edition, revised and enlarged.
20. **Essentials of Bacteriology.** By M. V. BALL, M. D. Fourth edition, revised.
21. **Essentials of Nervous Diseases and Insanity.** By JOHN C. SHAW, M. D. Third edition, revised.
22. **Essentials of Medical Physics.** By FRED J. BROCKWAY, M. D. Second edition, revised.
23. **Essentials of Medical Electricity.** By DAVID D. STEWART, M. D., and EDWARD S. LAWRENCE, M. D.
24. **Essentials of Diseases of the Ear.** By E. B. GLEASON, M. D. Second edition, revised and greatly enlarged.
25. **Essentials of Histology.** By LOUIS LEROY, M. D. With 73 original illustrations.

Pamphlet containing specimen pages, etc., sent free upon application.

Saunders' Medical Hand-Atlases.

VOLUMES NOW READY.

ATLAS AND EPITOME OF INTERNAL MEDICINE AND CLINICAL DIAGNOSIS.

By DR. CHR. JAKOB, of Erlangen. Edited by AUGUSTUS A. ESHNER, M. D., Professor of Clinical Medicine, Philadelphia Polyclinic. With 179 colored figures on 68 plates, 64 text-illustrations, 259 pages of text. Cloth, \$3.00 net.

ATLAS OF LEGAL MEDICINE.

By DR. E. R. VON HOFFMAN, of Vienna. Edited by FREDERICK PETERSON, M. D., Chief of Clinic, Nervous Department, College of Physicians and Surgeons, New York. With 120 colored figures on 56 plates and 193 beautiful half-tone illustrations. Cloth, \$3.50 net.

ATLAS AND EPITOME OF DISEASES OF THE LARYNX.

By DR. L. GRUNWALD, of Munich. Edited by CHARLES P. GRAYSON, M. D., Physician-in-Charge, Throat and Nose Department, Hospital of the University of Pennsylvania. With 107 colored figures on 44 plates, 25 text-illustrations, and 103 pages of text. Cloth, \$2.50 net.

ATLAS AND EPITOME OF OPERATIVE SURGERY.

By DR. O. ZUCKERKANDL, of Vienna. Edited by J. CHALMERS DACOSTA, M. D., Professor of Principles of Surgery and Clinical Surgery, Jefferson Medical College, Philadelphia. With 24 colored plates, 214 text-illustrations, and 395 pages of text. Cloth, \$3.00 net.

ATLAS AND EPITOME OF SYPHILIS AND THE VENEREAL DISEASES.

By PROF. DR. FRANZ MRACEK, of Vienna. Edited by L. BOLTON BANGS, M. D., Professor of Genito-Urinary Surgery, University and Bellevue Hospital Medical College, New York. With 71 colored plates, 16 illustrations, and 122 pages of text. Cloth, \$3.50 net.

ATLAS AND EPITOME OF EXTERNAL DISEASES OF THE EYE.

By DR. O. HAAB, of Zurich. Edited by G. E. DE SCHWEINITZ, M. D., Professor of Ophthalmology, Jefferson Medical College, Philadelphia. With 76 colored illustrations on 40 plates and 228 pages of text. Cloth, \$3.00 net.

ATLAS AND EPITOME OF SKIN DISEASES.

By PROF. DR. FRANZ MRACEK, of Vienna. Edited by HENRY W. STELWAGON, M. D., Clinical Professor of Dermatology, Jefferson Medical College, Philadelphia. With 63 colored plates, 39 half-tone illustrations, and 200 pages of text. Cloth, \$3.50 net.

ATLAS AND EPITOME OF SPECIAL PATHOLOGICAL HISTOLOGY.

By DR. H. DURCK, of Munich. Edited by LUDWIG HEKTOEN, M. D., Professor of Pathology, Rush Medical College, Chicago. In Two Parts. Part I. *Ready*, including Circulatory, Respiratory, and Gastro-intestinal Tract, 120 colored figures on 62 plates, 158 pages of text. Part II. *Ready Shortly*. Price of Part I., \$3.00 net.

Saunders' Medical Hand-Atlases.

VOLUMES JUST ISSUED.

ATLAS AND EPITOME OF DISEASES CAUSED BY ACCIDENTS.

By DR. ED. GOLEBIEWSKI, of Berlin. Translated and edited with additions by PEARCE BAILEY, M. D., Attending Physician to the Department of Corrections and to the Almshouse and Incurable Hospitals, New York. With 40 colored plates, 143 text-illustrations, and 600 pages of text. Cloth, \$4.00 net.

ATLAS AND EPITOME OF GYNECOLOGY.

By DR. O. SHAEFFER, of Heidelberg. *From the Second Revised German Edition.* Edited by RICHARD C. NORRIS, A. M., M. D., Gynecologist to the Methodist Episcopal and the Philadelphia Hospitals; Surgeon-in-Charge of Preston Retreat, Philadelphia. With 90 colored plates, 65 text-illustrations, and 308 pages of text. Cloth, \$3.50 net.

ATLAS AND EPITOME OF THE NERVOUS SYSTEM AND ITS DISEASES.

By PROFESSOR DR. CHR. JAKOB, of Erlangen. *From the Second Revised and Enlarged German Edition.* Edited by EDWARD D. FISHER, M. D., Professor of Diseases of the Nervous System, University and Bellevue Hospital Medical College, New York. With 83 plates and a copious text. \$3 50 net.

ATLAS AND EPITOME OF LABOR AND OPERATIVE OBSTETRICS.

By DR. O. SHAEFFER, of Heidelberg. *From the Fifth Revised and Enlarged German Edition.* Edited by J. CLIFTON EDGAR, M. D., Professor of Obstetrics and Clinical Midwifery, Cornell University Medical School. With 126 colored illustrations. \$2.00 net.

ATLAS AND EPITOME OF OBSTETRICAL DIAGNOSIS AND TREATMENT.

By DR. O. SHAEFFER, of Heidelberg. *From the Second Revised and Enlarged German Edition.* Edited by J. CLIFTON EDGAR, M. D., Professor of Obstetrics and Clinical Midwifery, Cornell University Medical School. 72 colored plates, numerous text-illustrations, and copious text. \$3.00 net.

ATLAS AND EPITOME OF OPHTHALMOSCOPY AND OPHTHALMOSCOPIC DIAGNOSIS.

By DR. O. HAAB, of Zurich. *From the Third Revised and Enlarged German Edition.* Edited by G. E. DE SCHWEINITZ, M. D., Professor of Ophthalmology, Jefferson Medical College, Philadelphia. With 152 colored figures and 82 pages of text. Cloth, \$3.00 net.

ATLAS AND EPITOME OF BACTERIOLOGY.

Including a Hand-Book of Special Bacteriologic Diagnosis. By PROF. DR. K. B. LEHMANN and DR. R. O. NEUMANN, of Wurzburg. *From the Second Revised German Edition.* Edited by GEORGE H. WEAVER, M. D., Assistant Professor of Pathology and Bacteriology, Rush Medical College, Chicago. Two volumes, with over 600 colored lithographic figures, numerous text-illustrations, and 500 pages of text.

ADDITIONAL VOLUMES IN PREPARATION.

Nothnagel's Encyclopedia OF PRACTICAL MEDICINE.

Edited by **ALFRED STENGEL, M.D.,**

Professor of Clinical Medicine in the University of Pennsylvania ; Visiting
Physician to the Pennsylvania Hospital.

IT is universally acknowledged that the Germans lead the world in Internal Medicine ; and of all the German works on this subject, Nothnagel's "Special Pathology and Therapeutics" is conceded by scholars to be without question the **best System of Medicine in existence**. So necessary is this book in the study of Internal Medicine that it comes largely to this country in the original German. In view of these facts, Messrs. W. B. Saunders & Company have arranged with the publishers to issue at once an **authorized edition** of this great encyclopedia of medicine in English.

For the present a set of some ten or twelve volumes, representing the most practical part of this encyclopedia, and selected with especial thought of the **needs of the practical physician**, will be published. These volumes will contain the **real essence** of the entire work, and the purchaser will therefore obtain at less than half the cost the cream of the original. Later the special and more strictly scientific volumes will be offered from time to time.

The work will be translated by men possessing thorough knowledge of both English and German, and **each volume** will be **edited by a prominent specialist** on the subject to which it is devoted. It will thus be brought thoroughly up to date, and the American edition will be more than a mere translation of the German ; for, in addition to the matter contained in the original, it will represent the **very latest views of the leading American specialists** in the various departments of Internal Medicine. The whole System will be under the editorial supervision of **Dr. Alfred Stengel**, who will select the subjects for the American edition, and will choose the editors of the different volumes.

Unlike most encyclopedias, the publication of this work **will not be extended over a number of years**, but five or six volumes will be issued during the coming year, and the remainder of the series at the same rate. Moreover, each volume will be revised to the date of its publication by the American editor. This will obviate the objection that has heretofore existed to systems published in a number of volumes, since the subscriber will receive the completed work while the earlier volumes are still fresh.

The usual method of publishers, when issuing a work of this kind, has been to compel physicians to take the entire System. This seems to us in many cases to be undesirable. Therefore, in purchasing this encyclopedia, physicians will be given the opportunity of subscribing for the entire System at one time ; but any single volume or any number of volumes may be obtained by those who do not desire the complete series. This latter method, while not so profitable to the publisher, **offers to the purchaser many advantages** which will be appreciated by those who do not care to subscribe for the entire work at one time.

This American edition of Nothnagel's Encyclopedia will, without question, form the **greatest System of Medicine ever produced**, and the publishers feel confident that it will meet with general favor in the medical profession.

NOTHNAGEL'S ENCYCLOPEDIA

VOLUMES JUST ISSUED AND IN PRESS

VOLUME I

Editor, William Osler, M. D.,
F. R. C. P.

Professor of Medicine in Johns Hopkins University

CONTENTS

Typhoid Fever. By DR. H. CURSCHMANN, of Leipsic. Typhus Fever. By DR. H. CURSCHMANN, of Leipsic.

Handsome octavo volume of about 600 pages.
Just Issued

VOLUME II

Editor, Sir J. W. Moore, B. A., M. D.,
F. R. C. P. I., of Dublin

Professor of Practice of Medicine, Royal College of Surgeons in Ireland

CONTENTS

Erysipelas and Erysipeloid. By DR. H. LENHARTZ, of Hamburg. Cholera Asiatica and Cholera Nostras. By DR. K. VON LIEBERMEISTER, of Tübingen. Whooping Cough and Hay Fever. By DR. G. STICKER, of Giessen. Varicella. By DR. TH. VON JÜRGENSEN, of Tübingen. Variola (including Vaccination). By DR. H. IMMERMAN, of Basle.

Handsome octavo volume of over 700 pages.
Just Issued

VOLUME III

Editor, William P. Northrup, M. D.

Professor of Pediatrics, University and Bellevue Medical College

CONTENTS

Measles. By DR. TH. VON JÜRGENSEN, of Tübingen. Scarlet Fever. By the same author. Rotheln. By the same author.

VOLUME VI

Editor, Alfred Stengel, M. D.

Professor of Clinical Medicine, University of Pennsylvania

CONTENTS

Anemia. By DR. P. EHRLICH, of Frankfort-on-the-Main, and DR. A. LAZARUS, of Charlottenburg. Chlorosis. By DR. K. VON NOORDEN, of Frankfort-on-the-Main. Diseases of the Spleen and Hemorrhagic Diathesis. By DR. M. LITTEN, of Berlin.

VOLUME VII

Editor, John H. Musser, M. D.

Professor of Clinical Medicine, University of Pennsylvania

CONTENTS

Diseases of the Bronchi. By DR. F. A. HOFFMANN, of Leipsic. Diseases of the Pleura. By DR. ROSENBACH, of Berlin. Pneumonia. By DR. E. AUFRECHT, of Magdeburg.

VOLUME VIII

Editor, Charles G. Stockton, M. D.

Professor of Medicine, University of Buffalo

CONTENTS

Diseases of the Stomach. By DR. F. RIEGEL, of Giessen.

VOLUME IX

Editor, Frederick A. Packard, M. D.

Physician to the Pennsylvania Hospital and to the Children's Hospital, Philadelphia

CONTENTS

Diseases of the Liver. By DRS. H. QUINCKE and G. HOPPE-SEYLER, of Kiel.

VOLUME X

Editor, Reginald H. Fitz, A. M., M. D.

Hersey Professor of the Theory and Practice of Physic, Harvard University

CONTENTS

Diseases of the Pancreas. By DR. L. OSER, of Vienna. Diseases of the Suprarenals. By DR. E. NEUSSER, of Vienna.

VOLUMES IV, V, and XI

Editors announced later

Vol. IV.—Influenza and Dengue. By DR. O. LEICHTENSTEIN, of Cologne. Malarial Diseases. By DR. J. MANNABERG, of Vienna.

Vol. V.—Tuberculosis and Acute General Miliary Tuberculosis. By DR. G. CORNET, of Berlin.

Vol. XI.—Diseases of the Intestines and Peritoneum. By DR. H. NOTHNAGEL, of Vienna.

CLASSIFIED LIST

OF THE

MEDICAL PUBLICATIONS

OF

W. B. SAUNDERS & COMPANY

ANATOMY, EMBRYOLOGY, HISTOLOGY.

Bohm, Davidoff, and Huber—A Text-Book of Histology,	4
Clarkson—A Text-Book of Histology,	5
Haynes—A Manual of Anatomy,	7
Heisler—A Text-Book of Embryology,	7
Leroy—Essentials of Histology,	15
Nancrede—Essentials of Anatomy,	15
Nancrede—Essentials of Anatomy and Manual of Practical Dissection,	10

BACTERIOLOGY.

Ball—Essentials of Bacteriology,	15
Frothingham—Laboratory Guide,	6
Gorham—Laboratory Course in Bacteriology,	22
Lehmann and Neumann—Atlas of Bacteriology,	17
Levy and Klemperer's Clinical Bacteriology,	9
Mallory and Wright—Pathological Technique,	9
McFarland—Pathogenic Bacteria,	9

CHARTS, DIET-LISTS, ETC.

Griffith—Infant's Weight Chart,	7
Hart—Diet in Sickness and in Health,	7
Keen—Operation Blank,	8
Laine—Temperature Chart,	9
Meigs—Feeding in Early Infancy,	10
Starr—Diets for Infants and Children,	12
Thomas—Diet-Lists,	13

CHEMISTRY AND PHYSICS.

Brockway—Essentials of Medical Physics,	15
Wolff—Essentials of Medical Chemistry,	15

CHILDREN.

An American Text-Book of Diseases of Children,	1
Griffith—Care of the Baby,	7
Griffith—Infant's Weight Chart,	7
Meigs—Feeding in Early Infancy,	10
Powell—Essentials of Diseases of Children,	15
Starr—Diets for Infants and Children,	12

DIAGNOSIS.

Cohen and Eshner—Essentials of Diagnosis,	15
Corwin—Physical Diagnosis,	5
Vierordt—Medical Diagnosis,	14

DICTIONARIES.

The American Illustrated Medical Dictionary,	3
The American Pocket Medical Dictionary,	3
Morton—Nurses' Dictionary,	10

EYE, EAR, NOSE, AND THROAT.

An American Text-Book of Diseases of the Eye, Ear, Nose, and Throat,	1
De Schweinitz—Diseases of the Eye,	6
Friedrich and Curtis—Rhinology, Laryngology, and Otology,	6
Gleason—Essentials of the Ear,	15
Gleason—Essentials of Nose and Throat,	15
Gradle—Ear, Nose, and Throat,	22
Grunwald and Grayson—Atlas of Diseases of the Larynx,	16
Haab and de Schweinitz—Atlas of External Diseases of the Eye,	16
Jackson—Manual of Diseases of the Eye,	8
Jackson—Essentials Diseases of Eye,	15
Kyle—Diseases of the Nose and Throat,	9

GENITO-URINARY.

An American Text-Book of Genito-Urinary and Skin Diseases,	2
Hyde and Montgomery—Syphilis and the Venereal Diseases,	8
Martin—Essentials of Minor Surgery, Bandaging, and Venereal Diseases,	15
Mracek and Bangs—Atlas of Syphilis and the Venereal Diseases,	16
Saundby—Renal and Urinary Diseases,	11
Senn—Genito-Urinary Tuberculosis,	12
Vecki—Sexual Impotence,	14

GYNECOLOGY.

American Text-Book of Gynecology,	2
Cragin—Essentials of Gynecology,	15
Garrigues—Diseases of Women,	6
Long—Syllabus of Gynecology,	9
Penrose—Diseases of Women,	10
Pryor—Pelvic Inflammations,	11
Schaeffer and Norris—Atlas of Gynecology,	17

HYGIENE.

Abbott—Hygiene of Transmissible Diseases,	3
Bergey—Principles of Hygiene,	22
Pyle—Personal Hygiene,	11

MATERIA MEDICA, PHARMACOLOGY, and THERAPEUTICS.

An American Text-Book of Applied Therapeutics,	1
Butler—Text-Book of Materia Medica, Therapeutics, and Pharmacology,	4
Morris—Ess. of M. M. and Therapeutics,	15
Saunders' Pocket Medical Formulary,	11
Sayre—Essentials of Pharmacy,	15
Sollmann—Text-Book of Pharmacology,	22
Stevens—Modern Therapeutics,	13
Stoney—Materia Medica for Nurses,	13
Thornton—Prescription-Writing,	13

MEDICAL JURISPRUDENCE AND TOXICOLOGY.

Chapman—Medical Jurisprudence and Toxicology,	5
Golebiewski and Bailey—Atlas of Diseases Caused by Accidents,	17
Hofmann and Peterson—Atlas of Legal Medicine,	16

NERVOUS AND MENTAL DISEASES, ETC.

Brower—Manual of Insanity,	22
Chapin—Compendium of Insanity,	5
Church and Peterson—Nervous and Mental Diseases,	5
Jakob and Fisher—Atlas of Nervous System,	17
Shaw—Essentials of Nervous Diseases and Insanity,	15

NURSING.

Davis—Obstetric and Gynecologic Nursing,	6
Griffith—The Care of the Baby,	7
Hart—Diet in Sickness and in Health,	7
Meigs—Feeding in Early Infancy,	10
Morten—Nurses' Dictionary,	10
Stoney—Materia Medica for Nurses,	13
Stoney—Practical Points in Nursing,	13
Stoney—Surgical Technic for Nurses,	13
Watson—Handbook for Nurses,	14

OBSTETRICS.

An American Text-Book of Obstetrics,	2
Ashton—Essentials of Obstetrics,	15
Boisliniere—Obstetric Accidents,	4
Dorland—Modern Obstetrics,	6
Hirst—Text-Book of Obstetrics,	7
Norris—Syllabus of Obstetrics,	10
Schaeffer and Edgar—Atlas of Obstetrical Diagnosis and Treatment,	17

PATHOLOGY.

An American Text-Book of Pathology,	2
Durck and Hektoen—Atlas of Pathologic Histology,	16
Kalteyer—Essentials of Pathology,	15
Mallory and Wright—Pathological Technique,	9
Senn—Pathology and Surgical Treatment of Tumors,	12
Stengel—Text-Book of Pathology,	12
Warren—Surgical Pathology,	14

PHYSIOLOGY.

American Text-Book of Physiology,	2
Budgett—Essentials of Physiology,	15
Raymond—Text-Book of Physiology,	11
Stewart—Manual of Physiology,	13

PRACTICE OF MEDICINE.

An American Year-Book of Medicine and Surgery,	3
Anders—Practice of Medicine,	4
Eichhorst—Practice of Medicine,	6
Lockwood—Practice of Medicine,	9
Morris—Ess. of Practice of Medicine,	15
Salinger & Kalteyer—Mod. Medicine,	11
Stevens—Practice of Medicine,	13

SKIN AND VENEREAL.

An American Text-Book of Genito-Urinary and Skin Diseases,	2
Hyde and Montgomery—Syphilis and the Venereal Diseases,	8
Martin—Essentials of Minor Surgery, Bandaging, and Venereal Diseases,	15
Mracek and Stelwagon—Atlas of Diseases of the Skin,	16
Stelwagon—Essentials of Diseases of the Skin,	15

SURGERY.

An American Text-Book of Surgery,	2
An American Year-Book of Medicine and Surgery,	3
Beck—Fractures,	4
Beck—Manual of Surgical Asepsis,	4
Da Costa—Manual of Surgery,	5
International Text-Book of Surgery,	8
Keen—Operation Blank,	8
Keen—The Surgical Complications and Sequels of Typhoid Fever,	8
Macdonald—Surgical Diagnosis and Treatment,	9
Martin—Essentials of Minor Surgery, Bandaging, and Venereal Diseases,	15
Martin—Essentials of Surgery,	15
Moore—Orthopedic Surgery,	10
Nancrede—Principles of Surgery,	10
Pye—Bandaging and Surgical Dressing,	11
Scudder—Treatment of Fractures,	12
Senn—Genito-Urinary Tuberculosis,	12
Senn—Practical Surgery,	12
Senn—Syllabus of Surgery,	12
Senn—Pathology and Surgical Treatment of Tumors,	12
Warren—Surgical Pathology and Therapeutics,	14
Zuckerkandl and Da Costa—Atlas of Operative Surgery,	16

URINE AND URINARY DISEASES.

Ogden—Clinical Examination of the Urine,	10
Saundby—Renal and Urinary Diseases,	11
Wolf—Handbook of Urine Examination,	22
Wolff—Examination of Urine,	15

MISCELLANEOUS.

Abbott—Hygiene of Transmissible Diseases,	3
Bastin—Laboratory Exercises in Botany,	4
Golebiewski and Bailey—Atlas of Diseases Caused by Accidents,	17
Gould and Pyle—Anomalies and Curiosities of Medicine,	7
Grafstrom—Massage,	7
Keating—Examination for Life Insurance,	8
Pyle—A Manual of Personal Hygiene,	11
Saunders' Medical Hand-Atlases,	16, 17
Saunders' Pocket Medical Formulary,	11
Saunders' Question-Compends,	14, 15
Stewart and Lawrence—Essentials of Medical Electricity,	15
Thornton—Dose-Book and Manual of Prescription-Writing,	13
Van Valzah and Nisbet—Diseases of the Stomach,	13

THE LATEST BOOKS.

Bergey's Principles of Hygiene.

The Principles of Hygiene: A Practical Manual for Students, Physicians, and Health Officers. By D. H. BERGEY, A. M., M. D., First Assistant, Laboratory of Hygiene, University of Pennsylvania. Handsome octavo volume of about 500 pages, illustrated.

Brower's Manual of Insanity.

A Practical Manual of Insanity. By DANIEL R. BROWER, M. D., Professor of Nervous and Mental Diseases, Rush Medical College, Chicago. 12mo volume of 425 pages, illustrated.

Gorham's Bacteriology.

A Laboratory Course in Bacteriology. By F. P. GORHAM, M. A., Assistant Professor in Biology, Brown University. 12mo volume of about 160 pages, fully illustrated.

Gradle on the Nose, Throat, and Ear.

Diseases of the Nose, Throat, and Ear. By HENRY GRADLE, M. D., Professor of Ophthalmology and Otology, Northwestern University Medical School, Chicago. Handsome octavo volume of 800 pages, profusely illustrated.

Sollmann's Pharmacology.

A Text-Book of Pharmacology. By TORALD SOLLMANN, M. D., Lecturer on Pharmacology, Western Reserve University, Cleveland, Ohio. Royal octavo volume of about 700 pages.

Wolf's Examination of Urine.

A Handbook of Physiologic Chemistry and Urine Examination. By CHARLES G. L. WOLF, M. D., Instructor in Physiologic Chemistry, Cornell University Medical College. 12mo volume of about 160 pages.