A LABORATORY GUIDE

IN

ELEMENTARY BACTERIOLOGY

BY

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PREFACE.

The following pages constitute, substantially, the material which has been furnished the students in Bacteriology at the University of Wisconsin, in mimeograph form, for several years. They contain directions for the performance of certain fundamental exercises in Bacteriology.

In a rapidly developing subject it is important that the various exercises be worded so as to lend themselves readily to changes which become desirable from time to time. With this end in view the exercises have been divided, where possible, into a general and a special part. The general directions contain the essential part of the exercise which does not permit of any considerable variation, while the special directions embrace such features as are most subject to modification, as for instance, the particular organism to be used, the kind of medium, the incubation temperature, etc. Desirable changes here are easily indicated when the exercise is assigned.

Some of the exercises can be performed in a few minutes, while others require several days for their completion. No attempt has been made to group them according to their length, nor to divide the text into lessons, but as far as possible they are arranged in the order in which they would be logically used in the laboratory.

The right hand pages have been left for notes and drawings with the idea that notes in permanent form are the only ones of value to the student in subsequent years.

The charts of the various organisms furnish a most satisfactory means for recording the observations made during the study of a germ and are especially convenient for reference.

Part I. is the work required of students taking the General Course in which special emphasis is placed on the biology of bacteria. It is completed in the first semester. Part II. which is given during the second semester includes the more specialized phases of the work, particularly as applied to the student preparing for medicine.

References have been made to all of the leading English text-books and occasionally to original sources. It is expected that the student will make constant use of these references.

My thanks are due Prof. H. L. Russell under whose general direction the work outlined here is given, for valuable help in the selection and arrangement of the material and for generous council. I am also indebted to Mr. E. G. Hastings, Assistant Bacteriologist to the Wisconsin Experiment Station, for critical reading of manuscript and proof.

WILLIAM DODGE FROST.

MADISON, WIS., January, 1901.

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LIST OF TEXTS AND REFERENCE WORKS WITH ABBREVIATIONS USED.

A.— Abbott: Principles of Bacteriology. Lea Bros. & Co. Philadelphia, 5th Edit., 1899.

B.— Bowhill: Manual of Bacteriological Technique. Oliver & Boyd, London, 1899.

F.— Fischer: Structure and Functions of Bacteria. Clarendon Press, New York, 1900.

Fr.— Frankland: Micro-organisms of Water. Longmans, Green & Co., 1894.

G. – Gage: The Microscope. Comstock Pub. Co., Ithaca, N. Y., 7th Edit., 1899.

H.— Hewlett: Manual of Bacteriology. Blakiston, Son & Co., Philadelphia, 1898.

J. H.— Jordan's Translation of Hueppe: Principles of Bacteriology. Open Court Pub. Co., Chicago, 1899.

v. J.— v. Jaksch: Clinical Diagnosis. Charles Griffin & Co., London, 4th Edit., 1899.

K. & D.— Kanthack & Drysdale: Practical Bacteriology. MacMillan Co., New York, 1895.

L.— Lafar: Technical Mycology. Vol. 1. Lippencott Co., Philadelphia, 1898.

L. & K.— Levy & Klemperer: Clinical Bacteriology. Saunders & Co., Philadelphia, 1900.

L. & N.— Lehmann & Neumann: Atlas and Essentials of Bacteriology. Wood & Co., New York, 1897.

M.— Moore: Laboratory Directions for Beginners in Bacteriology. Ginn & Co , New York, 1900.

M. & R.— Muir & Ritchie: Manual of Bacteriology. MacMillan Co , New York, 2nd Edit., 1899.

M. & W.—Mallory & Wright: Pathological Technique. Saunders & Co , Philadelphia, 1897.

McF.— McFarland: Text-Book of Pathogenic Bacteria. Saunders & Co., Philadelphia, 2nd Edit., 1898.

N.— Novy: Laboratory Work in Bacteriology. Geo. Wahr, Ann Arbor, Mich., 2nd Edit., 1899.

Ne.— Newman: Bacteria. Putnam, New York, 1899.

P.— Park: Bacteriology in Medicine and Surgery. Lea Bros. & Co., Philadelphia, 1899.

P. B. C — Proceedings of the Bacteriological Committee from Jour. Amer. Pub. Health Assn. Vol. XXII.

P. & M.— Peamain & Moor: Applied Bacteriology. Baillière, Tindall & Cox, London, 1897.

S.— Sternberg: Manual of Bacteriology. Wood & Co., New York, 1893.

Si.— Simon: Clinical Diagnosis. Lea Bros. & Co., Philadelphia, 2d Edit., 1897.

W.— Woodhead: Bacteria and Their Products. Charles Scribner & Sons, New York, 1892.

Wm.— Williams: Manual of Bacteriology. Blakiston, Son & Co., Philadelphia, 1898.

LIST OF APPARATUS.

This list comprises the apparatus which is to be under the exclusive control of the student and does not include the general laboratory outfit, such as sterilizers, incubators, microscopes, general chemical supplies, etc.

FOR INDIVIDUAL USE.

A.

50 ($\frac{1}{2}$ oz.) cover-glasses, 18 mm. ($\frac{3}{4}$ in.) square and 0.17 mm. thick (No. 2).
50 glass slides.
100 labels, 2 cm. square.
12 cm. platinum wire (No. 27).
1 pair cover-glass forceps (Cornet or Stewart).
1 pair fine pointed forceps.
2 slide boxes for 50 slides.
1 hanging-drop slide.
1 towel.

B.

1 flask, 1000 cc.
1 flask, 400 cc.
3 flasks, 250 cc.
1 flask, 100 cc.
200 test-tubes (15 × 120 mm.).
15 Petri dishes (10 cm).
2 fermentation tubes.
2 glass tumblers.
4 tin cans.
2 glass rods for platinum needles.
3 pipettes, 1 cc.
1 brass tube to hold pipettes (25 × 250 mm.).
8 stain bottles with pipettes, in block.
1 waste dish.
1 yard of muslin.
3 sheets of filter paper.
3 sheets of lens paper.

FOR GROUP USE (About Four Students).

1 glass funnel, 12 cm.
1 glass funnel, 5 cm.
1 filtering flask with rubber stopper.
2 stirring rods.
1 pipette, 5 cc.
1 thermometer, 0-100° C.
10 cm. rubber tubing. 1 cm. dia See Fig. 1.
1 Mohr stopcock.
1 anaerobic jar for plates.
1 anaerobic jar for tubes.
1 potato knife.
1 Bunsen burner with tubing.
1 piece of wire gauze.
1 tripod with reducing rings.
1 rice cooker.
3 small wire baskets.
1 enamel pan.
1 roll of cotton wool.
½ lb. absorbent cotton.
1 piece of Russia iron, 12 cm. square.
1 graduated cylinder, 300 cc.
1 graduated cylinder, 100 cc.
1 graduated cylinder, 25 cc.
1 evaporating dish, 10 cm.
1 disinfecting jar.
1 copper cup.
1 ring stand with clamp.
1 test-tube brush.

LABORATORY RULES.

I. Food should not be eaten in the laboratory and lead pencils or labels should not be moistened with the tongue.

II. All possible cleanliness should be observed in the care of apparatus, desk, etc.

III. The platinum needles used in making cultures should be sterilized shortly before and immediately after use and before they are laid down. When the needles are covered with infectious material they should be held at the side of the flame until dry before being sterilized; this will avoid the danger of scattering this material about the laboratory.

IV. If infectious matter should by accident come in contact with the hands or be dropped on the table or floor, corrosive sublimate (1:1000) should be immediately applied.

V. Solid material, culture media and corrosive sublimate should not be put in the sink but in crocks provided for the purpose. Burnt matches, pieces of paper, etc., should also be put in the crocks and not on the floor.

VI. All cultures of bacteria should be labeled with the name of the organism, the name of the student and the date.

VII. Discarded cultures should be covered with corrosive sublimate and placed in a proper receptacle and under no condition should they be left lying about the laboratory. Pipettes which have been used to handle infectious material should be placed in a glass cylinder containing a disinfectant or potassium bichromate and sulphuric acid.

VIII. When using the steam sterilizer see that there is enough water present before lighting the gas and do not leave the laboratory until the gas has been turned off.

IX. Before beginning an exercise read over the directions and look up some of the references. Keep notes of everything done and the conclusions reached on the right hand pages in this Guide. Make drawings wherever they will be of value. Outline with pencil and fill in with India ink. The laboratory Guide should be kept in the laboratory.

X. At the close of the day's work the tables should be washed with corrosive sublimate and the hands disinfected by washing in the sublimate solution (or a germicidal soap) and then in soap and water.

PART I.

GENERAL BACTERIOLOGY.

PART I.—GENERAL BACTERIOLOGY.

CHAPTER I.

MORPHOLOGY AND ELEMENTARY TECHNIQUE.

EXERCISE I. CLEANING GLASSWARE.

General Directions. All glassware to contain culture media must be thoroughly clean. New glassware should be washed in hot soap-suds (a test-tube brush will be needed for the test-tubes), rinsed in tap water and then placed for a few minutes in water to which about 1% of hydrochloric acid has been added to remove free alkali frequently present on new glass, and then thoroughly rinsed in tap water. It is then allowed to drain. Test-tubes and flasks are best dried by placing them on a drain board especially prepared, or standing them mouth down in a box with a cloth bottom or on filter paper.

Glassware containing media (discarded cultures, etc.), is best cleaned by first standing in water for some hours, or by being steamed and pouring out the material while in a liquid condition and then cleaning as above with the exception of the use of the hydrochloric acid.

References. A. 120; H. 39; K. & D. 81; M. & W. 74; N. 158; P. 223.

Special Directions. Clean as directed above, all flasks, test-tubes, fermentation tubes and Petri dishes in your possession.

EXERCISE II. PLUGGING FLASKS AND TUBES.

General Directions. When the flasks, test-tubes and fermentation tubes are thoroughly dry they are to be plugged with cotton. The cotton for this purpose should be of the best non-absorbent quality, i. e., as free from foreign matter as possible. The plugs should be sufficiently loose to permit the interchange of gases and at the same time tight enough to support the weight of the vessel and its contents, to prevent their being pulled out in handling the vessel. The cotton should be rolled into a cylinder of the proper diameter and long enough to extend into the mouth about 2½ cm. (1 in.) and project sufficiently to protect the lips from dust. The plug should be pushed in straight and not twisted; the surface next to the glass must be perfectly smooth, presenting no creases for the entrance of dust.

References. A. 121; H. 39; M. & W. 74; M. & R. 56; McF. 107; P. 223.

Special Directions. Plug all test-tubes, flasks and fermentation tubes in your possession.

EXERCISE III. STERILIZATION OF GLASSWARE.

General Directions. The glassware thus prepared is ready for sterilization, which process is accomplished in an apparatus called the *hot air sterilizer*. This is a sheet iron or copper box with a double wall which permits of rapid heating. The apparatus should

be so arranged that a temperature of 150° C. can be quickly reached and readily maintained. In such a sterilizer all glassware to be used for the reception of culture media, such as flasks, test-tubes, Petri dishes, etc., is submitted to a temperature of 140–150° C. *for 1 hour*, or until the cotton plugs are slightly browned; this change being due to the incipient charring of the cotton. The test-tubes are placed erect in square baskets made of galvanized iron wire. When the air in the sterilizer has cooled to about 40° C. the glassware can be taken out and stored ready for use. The Petri dishes are not to be opened until used for culture purposes.

References. A. 71 and 121; H. 32; L. & K. 74; M. & R. 36; N. 159; McF. 106; P. 223; S. 51.

Special Directions. All glassware prepared in I. is to be sterilized for one hour at 150° C. The small pipettes should be placed in brass tubes, provided for the purpose, and also sterilized.

EXERCISE IV. PREPARATION OF BOUILLON.

General Directions. Any one of the three methods (A B or C) may be used. They are arranged in order of preference, but method C is the most convenient, and hence most used.

A.	B.	C.
a. From 500 grams (1¼ lb.) of lean beef, remove the fat and connective tissue and mince (Hamburg steak). *b*. Add 1 liter of distilled water and after thoroughly shaking set in ice chest for 12 to 24 hours. *c*. Squeeze through a cloth and add enough distilled water to make 1 liter and place in vessel to cook. This may be done either in a flask which is heated in a water-bath or a sterilizer, or in a rice cooker. In this case use a 50% solution of calcium chloride in outer vessel instead of water as by this means the contents of the inner vessel can be brought to a rapid ebullition, something impossible by the use of water alone. *d*. Boil ½ hour and make up loss of water.	Secure meat as under A *a*, add 1 liter of distilled water, weigh (see *e* below), cook for ½ hour at about 70° C., and proceed as directed under *e* below.	Weigh out three grams of beef extract (such as Liebig's), add 1 liter of water, and then proceed as directed under *e* below.

e. Add to any of the above solutions:

1% (10 gms.) peptone (Witte) and ½% (5 gms.) common salt (NaCl), then weigh solution, with vessel, so that the water which is subsequently driven off in cooking can be accurately replaced.

f. Heat until ingredients are in solution, then restore the water lost by evaporation.

g. Neutralize or render slightly alkaline. *This is a very important step and calls for great care.* Method A is more accurate and should be employed for special or research work. For ordinary routine work B may be employed.

A.

1.) Titrate as follows: Pipette off 5 cc. of the fluid into a 4-inch evaporating dish, add 45 cc. of distilled water, boil for three minutes, add 1 cc. of phenolphthalein (0.5% substance in 50% alcohol), and then carefully run in, drop by drop, from a burette a twentieth normal *solution of sodium hydroxide ($\frac{n}{20}$Na OH) until the solution turns a faint pink color. Treat two other samples in the same way. If the amount of Na OH required is approximately the same in each case the average can be taken as the amount necessary to neutralize 5 cc. Calculate the amount necessary to neutralize the whole (1000–15 cc.). Since this amount would dilute the medium too much, a stronger solution (normal) is used, hence,

2.) Neutralize by adding $\frac{1}{20}$th of the volume calculated above of a normal solution of sodium hydroxide. Test the accuracy of the work at this point by the addition of a few drops of phenolphthalein to a cc. or so of the medium. If a faint pinkish tint is not obtained, titration and neutralization must be repeated.

B.

Use a normal solution of sodium hydroxide ($\frac{n}{1}$NaOH). Add to the hot solution a few cc. at a time, at first, later a few drops, stirring thoroughly with a glass rod. After each addition, test by placing a drop of the solution by means of the glass rod on a strip of red litmus paper, and then moisten the paper with distilled water. The addition should continue until the red litmus paper is turned blue, but no change occurs on blue litmus paper.

If by mistake more alkali is added than is required, the reaction can be corrected by the use of a normal solution of hydrochloric acid.

h. Boil for 5 minutes and restore weight.

i. Test reaction and adjust if necessary.

j. Add 0.5 to 1.5% of a normal hydrochloric acid if neutralized by method A, otherwise omit. The amount of acid to be added varies with the purpose for which the medium is to be used, e. g., in water analyses +1.5 (acid) is preferable, with the pathogenic bacteria a smaller amount of acid (+ 0.5) more nearly meets requirements.

k. Filter through moistened filter paper (Abbott p. 96), or absorbent cotton, (VII. *m*). If the filtrate is not perfectly clear, cool to 60° C., add the white of an egg, thoroughly mix and *boil for 5 minutes without stirring*.

The filtrate (bouillon) should be of a light straw color, perfectly clear, and should not give a precipitate on boiling.

References. A. 90; M. & R. 43; McF. 124; N. 234; P. 212; P. B. C. 18–24.

Special Directions. Prepare 1 liter of bouillon according to method C. Secure and put to soak meat for VII.

EXERCISE V. FILLING TEST-TUBES AND FLASKS WITH CULTURE MEDIA.

General Directions. In filling tubes be careful not to allow the media to touch the neck of the vessels as this will cause the cotton to stick to the glass when the plugs are removed. Place the culture fluid to be tubed in a funnel arranged with a delivery

* Normal solutions are prepared so that one liter at 16° C. shall contain the hydrogen equivalent of the active reagent weighed in grams (Sutton). For present purposes a 4 % solution of sodium hydrate is sufficiently accurate.

tube and stopcock (fig. 1), from which it can be run into sterile vessels. Test-tubes should contain 6-10 cc. of medium (about 3 cm. deep). Flasks are to be filled about three-fourths full.

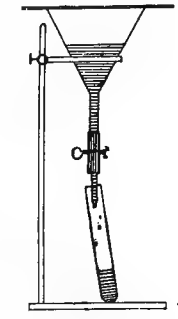


FIG. 1. Apparatus for filling test-tubes.

SPECIAL DIRECTIONS. Fill 15 test-tubes and preserve remainder of bouillon in larger flasks.

EXERCISE VI. STERILIZATION OF CULTURE MEDIA.

EXPLANATORY. To accomplish this steam is used almost exclusively either as streaming steam or under pressure. The unconfined steam is applied in an apparatus known as a steam sterilizer. Of the various patterns the Arnold is perhaps the most satisfactory. It is effective, economical in the use of gas, and does not allow the escape of large quantities of steam into the room, as a large part is condensed to be reconverted into steam. For student use the form shown in fig. 2 is very convenient. The method of using these different forms is identical. Always have plenty of water present before heating. The discontinuous method is most frequently employed. Exposure is made on three consecutive days for 20 minutes, beginning to count time when the material reaches the temperature of the steam, which will vary with different substances and the volume treated. Between successive steamings culture media should be kept under conditions favorable to bacterial development (room or incubator temperature).

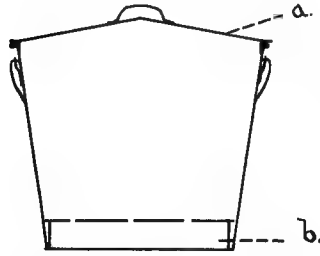


FIG. 2. Simple sterilizer consisting of a galvanized iron pail with a cover *a* and a false bottom *b*.

For the employment of steam under pressure the autoclave is essential. The lid should contain a thermometer as well as a steam gauge, safety and outlet valve. A thermo-regulator is also desirable. The following table gives the temperature corresponding to atmospheres of pressure:

Atmospheres.	*Degrees C.*
1	100
1.5	112.2
2	**121.4**
2.5	128.8
3	135.1

This table is only true when all of the air in the apparatus is replaced by steam, and *hence the steam must be allowed to escape freely before the outlet valve is closed.* A single exposure of 20 minutes at a temperature of 120° C. (one additional atmosphere) is sufficient to kill all germ life. After the proper exposure, care must be taken not to allow the steam to escape too rapidly, otherwise the culture media may be forced against the plugs owing to the unequal pressure.

GENERAL DIRECTIONS. Ordinary media may be sterilized by either method. Sugar media cannot be sterilized in the autoclave as it must not be heated above 100° C. The solidifying property of gelatin is impaired if submitted to a temperature of 120° C. longer than 15 minutes, and at a temperature above 120° C. momentarily.

REFERENCES. A. 55–73; M. & R. 37; McF. 109; N. 161; P. 213.

SPECIAL DIRECTIONS. Sterilize bouillon prepared in IV. for 20 miuutes in a steam sterilizer on three consecutive days.

N. B. *Some time is required to raise the temperature of the media to that of the steam,* especially if the vessels are large.

All media should be carefully examined every day for a week or more, and if "specks" or the least cloudiness appears, the medium is not sterile and the process of sterilization must be repeated.

All receptacles containing media should be labeled after sterilization. For this purpose labels can be purchased, the size used for glass slides, or gummed paper in sheets can be cut into squares (2 cm.). The labels are to be attached to each vessel 1 cm. from the lip. The kind of medium and the date of preparation should be written across the top, as BOUILLON 10-15-'99 leaving the rest of the label to be filled in when the medium is inoculated.

EXERCISE VII. PREPARATION OF GELATIN.

GENERAL DIRECTIONS.

a to *d*. Same as bouillon. (IV.)

e. Add 1% peptone; 0.5% salt and 10–15% * of best white gelatin, and weigh.

f. Heat until ingredients are dissolved.

g. Neutralize.

h. Boil 5 minutes and restore weight.

i. Test reaction.

j. If neutralized by method A add 5 cc. of a normal hydrochloric acid. In method B omit acid.

k. Cool and add egg and boil 5 minutes.

l. Filter. Arrange the apparatus shown in fig. 3. Use absorbent cotton. The funnel and flask should first be heated with warm water. Start the filter pump before pouring in the culture medium. This prevents the unfiltered gelatin from passing between the cotton and the glass.

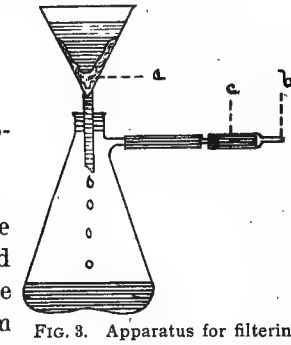


FIG. 3. Apparatus for filtering media through absorbent cotton; *a*, layer of cotton; *b*, tubes for making connection with air pump; *c*, Bunsen valve to prevent entrance of water into flasks.

m. Tube. (V.)

n. Sterilize.

o. Label.

REFERENCES. A. 95; H. 42; M. & R. 46; McF. 127; N. 153; P. B. C. 26.

SPECIAL DIRECTIONS. Make 1 liter, using method A. Fill 30 test-tubes. Put the remainder in flasks, sterilize in steam sterilizer or autoclave. Remember *long exposure to high heat* injures the solidifying properties of gelatin.

EXERCISE VIII. PREPARATION OF AGAR.

GENERAL DIRECTIONS.

a. Add 15 grams of agar-agar threads (finely chopped) to 500 cc. of water and either (1) boil until the agar-agar is dissolved (about ½ hour) and make up loss of water by evaporation, or (2) dissolve in autoclave by heating up to 120° C., closing off gas and allowing to cool.

* The amount to be varied according to the season of the year, 10 per cent in winter, 12–15 per cent in summer, but it should be remembered that different quantities affect the appearance of the culture.

b. 1)–4) Same as *a-d* in the preparation of bouillon (IV.), except that only one-half (500 cc.) of the amount of water is added to the beef or extract.

5) Add 1% peptone and 0.5% salt.

6) Heat until peptone is dissolved.

7) Neutralize.

8) Mix *a.* and *b.* (in case beef is used it will be necessary to cool *a.* to about 60° C. before mixing).

9) Boil 5 minutes and restore weight.

10) Test reaction.

11) Addition of egg will be necessary only where extract is used.

12) Filter as in case of gelatin, (IV. *m.*)

13) Tube.

14) Sterilize in steam for 15 minutes on three successive days or in autoclave for 20 minutes at 120° C. After the last sterilization place most of the tubes in a sloping position to harden (fig. 4), these are known as *agar slopes.* Those solidified horizontally can be used for plate cultures.

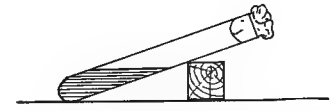


FIG. 4. Method of sloping agar.

15) Label.

REFERENCES. A. 100; H. 43; M. & R. 48; McF. 129; N. 235; P. B. C. 27; S. 43; Journal of Applied Microscopy, 1898, 1; 106.

SPECIAL DIRECTIONS. Use meat extract, make 1 liter, fill 25 tubes and after last sterilization incline 20 of them. Place the remainder in flasks and sterilize.

EXERCISE IX. PREPARATION OF POTATOES. (BOLTON.)

GENERAL DIRECTIONS.

a. Select a number of rather large test-tubes (150×20 mm.) place a small wad of absorbent cotton in the bottom of each (fig. 5 *a*), plug and sterilize as usual.

b. Wash a large potato, then with a cork borer slightly smaller than the test-tubes punch out cylinders about 5–6 cm. long.

c. Divide these diagonally and trim to shape indicated in fig. 5 *b.*

d. Add a few drops of distilled water to each test-tube and place pieces of potato in position.

e. Sterilize on three consecutive days for 30 to 45 minutes.

Unless the tubes are to be used immediately, they should be sealed. (XI.) The dark color can be prevented by immersing the pieces between *c* and *d* in running water for from 12–18 hours.

FIG. 5. Bolton's potato tube.

REFERENCES. A. 104; M. & R. 54; McF. 134; N. 183; P. 216; P. B. C. 28; S. 47.

SPECIAL DIRECTIONS. Prepare 15 test-tubes of potato, sterilize, label, and seal with paraffin. (XI. 2.)

EXERCISE X. PREPARATION OF WATER-BLANKS.

GENERAL DIRECTIONS. Water-blanks are prepared by placing exactly 10 cc. of a physiological salt solution (6 gms. per 1,000 cc. of water) in test-tubes and sterilizing in autoclave 15 minutes at 120 ° C., or in steamer 15 minutes on three successive days.

SPECIAL DIRECTIONS. Prepare and sterilize 10 water-blanks.

EXERCISE XI. CARE OF CULTURE MEDIA.

When sterile culture media (or test-tube cultures) are to be kept for some time they must be protected from evaporation and stored in a dark, cool place. Evaporation may be checked to a considerable extent, (1) by storing them in tin cans, e. g. quinine cans. Care must be taken, however, that these do not become too damp, in which case the mould fungi frequently grow through the cotton plugs; (2) flasks and test-tubes may be sealed by removing the plugs, dipping same in melted paraffin (melting point about 50° C.) and then replacing them; (3) by cutting off the projecting cotton and drawing over the mouth of the vessel a rubber cap (made for the purpose) which has been sterilized in a solution of mercuric bichloride (1: 1,000, spoken of in the laboratory as "sublimate solution"); or (4) By use of a cap of tin-foil. In this case the foil should be put on as soon as the tubes are filled, and sterilized with the medium.

All media should be carefully examined every day for a week or more, and if spots or the least cloudiness appears, the medium is not sterile and the process of sterilization must be repeated.

EXERCISE XII. PLATINUM NEEDLES.

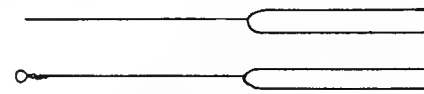


Fig. 6. Platinum needles.

General Directions. These are made by fusing a piece of No. 27 platinum wire (5 cm. long) into a glass rod or tube (18 cm. long). (Fig. 6.) Each student should have two such needles; in one the wire should be straight (designated "needle") and the other bent to form a "loop". This loop should be formed around a No. 10 wire. These instruments must be sterilized shortly before and immediately after use by heating the wire to a glow in the gas flame. The handle should also be passed through the flame two or three times. Cool before using. If the habit of sterilizing is thoroughly acquired much trouble will be avoided and possible danger prevented. These needles will be in constant use.

References. A. 125; M. & R. 58; N. 172; P. B. C. 33, foot note.

EXERCISE XIII. TEST-TUBE CULTURES.

Explanatory. The extreme minuteness and slight variation in the form of different bacteria render a thorough study of them by direct microscopic observation a difficult and well nigh impossible task. In their study, therefore, it is necessary to depart from the usually accepted rules that govern the determination of the life history of other forms of life and resort to special methods. The most successful of these are those known as culture methods. According to these methods the bacteria are sown on various food substances and upon these they develop forming masses easily visible to the naked eye. The manner of their growth and the changes which they produce in these media make it possible to detect differences which would otherwise escape attention. The most common culture media, bouillon, gelatin, agar and potato have already been prepared, and others will be described as needed.

Cultures may be made either in test-tubes (streak or stab cultures), or on glass plates, as plate cultures. The plate culture is especially important and is used (*a*) to obtain pure cultures; and (*b*) for ascertaining the character of the colonies as an aid to

their diagnosis. The tube-cultures are serviceable in giving opportunity for a further study of the characters as well as to furnish the most convenient method of maintaining the cultures.

General Directions. Bacteria when obtained in "pure culture" are usually grown in test-tube cultures. To make these a small portion of a previous culture is transferred to fresh culture media by means of the platinum needles.

a. Stab Cultures are made in test-tubes containing solid, transparent media, such as gelatin and agar. The end of a sterile needle is infected with the material to be transferred. The needle is then thrust into the medium to the bottom of the test-tube and withdrawn. In this way the bacteria are left along the entire length of the needle track. For method of holding tubes see fig. 7. They are held in an inclined position to prevent the possibility of infection.

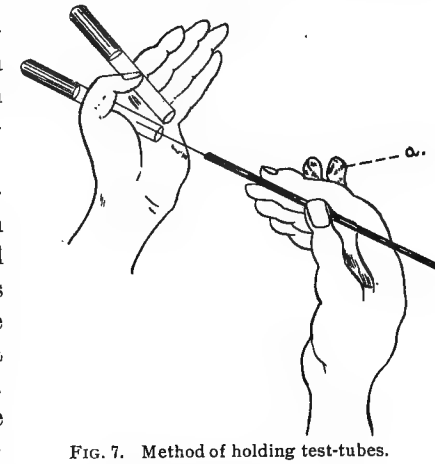


Fig. 7. Method of holding test-tubes.

b. Streak Cultures are cultures made by drawing the needle or loop over the surface of the medium (test-tubes with media having sloped surfaces or plate cultures). Agar, potato and blood serum are frequently used in this way, and occasionally gelatin.

c. Fluid Cultures (bouillon, milk, etc.), are inoculated by transferring the desired material to them on either the needle or loop.

References. A. 146; H. 51; M. & R. 60; McF. 146.

Special Directions.

a. Make a gelatin stab, an agar streak, a potato streak, and a bouillon culture of *Bacillus subtilis* (Ehrenb.) Cohn (hay bacillus) and *Bacillus coli* (Esch.) Mig. (colon bacillus) from agar cultures supplied.

b. Label *each tube*, writing the name of the *organism*, the *date* of inoculation and *your own name.*

c. Place the gelatin in the cool chamber, and the other cultures in the incubator at 28° C. (XIV).

EXERCISE XIV. INCUBATION OF CULTURES.

Explanatory. Most bacteria grow at ordinary temperatures (22° C.), but their growth is usually hastened by a higher temperature (e. g. 28°–30° C.). The pathogenic, or disease-producing bacteria grow best at the temperature of the human body (38° C.). All bacteriological laboratories are, therefore, supplied with apparatus arranged for maintaining constant temperatures, known as thermostats or incubators.

The non-pathogenic cultures are usually kept at 28° C., while the pathogenic ones are kept at 38° C. *All gelatin cultures, however, must be kept at a temperature several degrees below the melting point of gelatin*, i. e., not above 22° C. Ordinarily the temperature of the locker, especially near the floor, will be found satisfactory. In a very warm room, particularly in the summer, an artificially cooled chamber will be necessary.

Test-tube cultures are stored in the various incubators in tin cans or glass tumblers with a layer of cotton in the bottom, while the Petri dishes are stacked in low piles.

References. A. 136; H. 48; M. & R. 88; N. 178 & 243; P. 231; P. & M. 37.

SPECIAL DIRECTIONS.

a. Incubate all cultures of the non-pathogenic bacteria at 28° C., *except the gelatin.* Keep these in the cool chamber. After growth has taken place, the cultures can be taken from the incubator and kept at the room temperature.

b. Study and make diagrams of an incubator, a Reichert thermo-regulator, a Roux thermo-regulator and Koch's safety burner.

EXERCISE XV. CLEANING SLIDES AND COVER-GLASSES.

GENERAL DIRECTIONS. Slides can be sufficiently cleaned by washing in water or alcohol and drying with a towel. The cover-glasses for bacteriological work, however, must not only be freed from visible dirt but must be rendered free from fat. One of the best methods is the following: New cover-glasses are cleaned by washing in water and drying from alcohol between driers (two blocks 20x10x2½ mm. covered with several layers of cotton cloth or chamois skin), and then heating them on a piece of sheet iron or in hot air sterilizer for one hour at about 200° C. They are best kept in a clean Petri dish and handled with forceps. (Novy). Old slides and covers having balsam on them should first be dropped one by one into a cleaning solution (potassium bichromate 60, sulphuric acid 60, water 1000), and boiled for one-half hour and then treated as above.

SPECIAL DIRECTIONS. Clean ½ oz. of cover-glasses and place them in a clean Petri dish.

EXERCISE XVI. PREPARATION OF STAINING SOLUTIONS.

GENERAL DIRECTIONS. The dyes most useful for staining bacteria are the basic anilin dyes which come in powdered or crystalline form. (Gruebler's dyes are standard.) Those in most common use are Fuchsin, Methylen blue, Gentian violet and Bismark brown. They keep well in powdered form, with perhaps the exception of Methylen blue, but because of greater convenience and equally good keeping qualities, saturated alcoholic solutions are kept in stock. These are made by adding the dry dye to 95% alcohol to saturation and filtering. This form *can not be used for staining bacteria.* The following solutions are required to begin work with:

1. Aqueous solution of Gentian violet.
   - Saturated alcoholic solution of Gentian violet, - - - 2.5 cc.
   - Distilled water, - - - - - - 47.5 cc.
2. Saturated aqueous solution of Bismark brown.
3. Ziehl's carbol-fuchsin.
   - Saturated alcoholic solution of Fuchsin. - - - - 5 cc.
   - Solution of carbolic acid (5%)- - - - - - 45 cc.
4. Loeffler's Methylen blue.
   - Saturated alcoholic solution of Methylen blue, - - - 15 cc.
   - Potassium hydrate (1:10,000), - - - - - 50 cc.
5. Ehrlich's Anilin Oil Gentian violet.
   - Saturated alcoholic solution of Gentian violet, - - - 6 cc.
   - Absolute alcohol, - - - - - - - 5 cc.
   - Anilin water, - - - - - - - - 50 cc.

Anilin water is prepared by adding 2–3 cc. of anilin oil drop by drop to 50 cc. of water, thoroughly shaking and then filtering through moistened filter paper until perfectly clear.

This stain should stand 24 hours and then be filtered. It does not keep well and must not be used when more than 14 days old.

6. Gram's Iodine solution.

Iodine,	1 gm.
Potassium iodide,	2 gm.
Distilled water,	300 cc.

7. Gabbett's Methylen blue solution.

Methylen blue (dry),	2 gms.
Sulphuric acid,	25 cc.
Distilled water,	75 cc.

8. Alcohol, 96%.

References. A. 156; H. 75; M. & W. 245; M. & R. 103; McF. 90; P. 200.

Special Directions. Prepare the solutions of dyes from the saturated alcoholic solutions (furnished) and place them in 2 oz. bottles arranged with pipettes and neatly labeled. The bottles are conveniently kept in a block. Fig. 8.

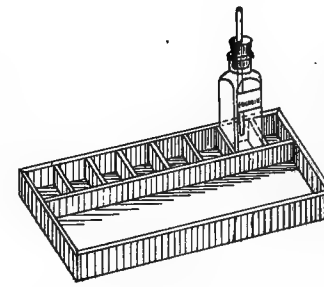


Fig. 8. Block for stain bottles.

EXERCISE XVII. SIMPLE COVER-GLASS PREPARATION.

General Directions. Bacteria may be studied under the microscope in a living condition in a hanging drop preparation (XIX); but on account of their hyaline character, which makes the examination difficult, the student should first learn to stain them and later make the hanging drop preparation. With a few exceptions all bacteria can be stained by the following process: A *small* drop of distilled water is placed on a clean cover-glass by means of the platinum loop. With a sterile *needle* a portion of the material to be examined is secured and while the cover-glass is held in the fingers of the left hand the bacteria on the needle are introduced into the water, thoroughly mixed and then spread in a thin film over as much of the surface of the cover-glass as possible. When the bacteria are taken from fluid media a drop of water will not be necessary. In this case use a *loop*. The film is now allowed to dry. If the drop is sufficiently small this will be a short process. It may be hastened by holding the cover-glass high over the flame, but it should always be held in the hand to prevent over-heating, which spoils the preparation.

When the film is thoroughly dry place the cover-glass in a pair of Cornet or Stewart forceps and "fix" the bacteria in the flame. This is done by passing the preparation through the upper portion of a gas flame, film side up. Three passages should be made, each consuming about one second of time. The forceps are now placed on the table and the film flooded with one of the anilin dyes. After the stain has acted for five or ten minutes it is washed off into a waste dish with a stream of distilled water, and while the cover-glass is still wet it is placed, bacteria side down, on a clean glass slide, being careful to avoid air bubbles. The surplus water is then taken up by means of a small piece of blotting or filter paper.

The preparation is now ready for microscopical examination. (For directions see XVIII).

The preparation can be made permanent either by allowing the water under the cover-glass to dry before it is removed, or by floating it off with water and afterwards drying. When dry a drop of Canada balsam, dissolved in xylene, is placed on the cover-glass and this is then lowered on to the slide again.

Resume.

a. Spread film,
b. Air dry,
c. Fix,
d. Stain,
e. Mount,
f. Examine,
g. Mount in balsam, or,
f. Mount in balsam,
g. Examine.

"The great mistake made by beginners is to take too much growth." (M. & R.)

REFERENCES. A. 151; H. 71; L. & K. 104; M. & W. 89; M. & R. 95; McF. 91; N. 147; P. 198; P. B. C. 11; S. 25.

SPECIAL DIRECTIONS. Make cover-glass preparation from agar streak of *B. subtilis* (XIII) staining with an aqueous solution of gentian violet for 5 minutes.

EXERCISE XVIII. USE OF MICROSCOPE.

GENERAL DIRECTIONS. For bacteriological purposes a microscope with a magnifying power of at least 500 diameters is needed. There should be a coarse adjustment (rack and pinion) as well as a fine micrometer screw; and the following accessories: two eye pieces, one 1 in. (25 mm.) and one 2 in. (50 mm.); three objectives, one $\frac{2}{3}$ in. (16 mm.), one $\frac{1}{6}$ in. (4 mm.), or $\frac{1}{8}$ in. (3.5 mm.) and one oil immersion $\frac{1}{10}$ in. or $\frac{1}{12}$ in. (2 mm.); a triple nose-piece, and an Abbe substage condenser with iris diaphragm mounting.

In the use of the microscope the following points should be noted:

a. LIGHT. The proper angle at which the mirror should be placed is best determined by removing the eye-piece and so arranging the mirror that the unobstructed light from the window covers the whole field. The ideal light is that from a white cloud. *Direct sunlight should never be used.*

b. ABBE CONDENSER. The purpose of the condenser is to furnish a large cone of light, and as it is corrected for parallel rays the *plane side of the mirror should always be used*, except when artificial light is employed. When highly stained objects are to be examined, *the open diaphragm should be used, but when the structural rather than the color picture is desired, it will be necessary to diminish the light by closing the diaphragm.* When the high powers are employed, raise the condenser as high as possible; for low powers a lower position will give better definition.

c. FOCUSING. Turn the proper objective in place and rack down until the objective nearly touches the cover-glass. *This should be done while the eye is held at one side and directs the movement.* Then with the eye at the tube slowly move up with the micrometer screw. *Never rack down with the eye at the tube.*

d. USE OF OIL-IMMERSION. The oil-immersion objective is indispensable to the proper study of bacteria. It is constructed upon the principle that a drop of fluid having the same refractive index as the objective, prevents the dispersion of light, thus permitting the use of lenses having a greater numerical aperture and longer working distance for

the same degree of amplification than is possible with the dry system. In using an immersion lens, place a small drop of oil on the preparation, then carefully lower the objective until it touches the oil drop and nearly touches the cover-glass. Apply eye to the ocular and focus upward very slowly with fine adjustment until the definition is clear. At the close of the day's work the oil must be removed from the objective and cover-glass. This is best accomplished by wiping them with a piece of Japanese paper made for the purpose. In case the oil should accidentally dry on the objective, it can be removed by adding a little more oil and allowing it to stand for a few minutes; it can then be wiped off with paper. If this method does not succeed, the objective should be taken to the instructor. Great care must be observed since solvents of the oil are also solvents for the lens mountings.

REFERENCES. See Gage; A. 190; H. 104; M. & R. 93; McF. 86; N. 123; P. 206.

SPECIAL DIRECTIONS.

a. Examine cover-glass preparations made in (XVII) first with $\frac{1}{6}$ in. objective, and then with the oil-immersion objective. If the specimen is satisfactory wipe off the oil and mount in Canada balsam.

b. Practice making cover-glass preparations by staining specimens from each of your cultures. Use Loeffler's methylen blue for the gelatin and bouillon; aqueous solution of gentian violet for agar, and carbol-fuchsin for potato. Examine, mount permanently and hand to instructor for inspection.

EXERCISE XIX. HANGING-DROP PREPARATIONS.

GENERAL DIRECTIONS. These are made by adding a small portion of bacterial culture from solid media to a drop of water on a clean cover-glass, or in case of fluid media by placing a loop of the culture medium on the cover-glass. A hollow ground glass slide having the rim of the cavity previously coated with vaseline, is inverted and lowered over the cover-glass enclosing the drop. With a careful, quick movement the preparation is now brought right side up.

Instead of the hollow ground glass-slide an ordinary glass-slide to which a small section of a glass or rubber tube has been cemented can be used, and in some cases is preferable.

In examining the preparation under a microscope focusing is a somewhat difficult process and must be carried out with great care. *Use a narrow diaphragm.* Find the edge of the drop with the low power ($\frac{2}{3}$ in. objective) adjusting slide so that edge of drop passes through the center of the field; then turn on the high power ($\frac{1}{6}$ in. objective) and focus *without moving the slide.* The edge of the drop is selected because the bacteria are here nearest the cover-glass and hence more easily focused upon than where they are deeper in the drop.

REFERENCES. A. 195; H. 101; L. & K. 102; M. & W. 111; M. & R. 94; McF. 88; N. 142; P. 209.

SPECIAL DIRECTIONS.

a. Make hanging-drop preparation of *B. subtilis* from agar or bouillon. (XIII)

b. Make same preparation of *B. coli.* (XIII)

c. Make same preparation of organism supplied. (Micrococcus)

d. Make same preparation of water containing particles of india ink or carmine in suspension. Study character of movement in all cases. Distinguish between vital and molecular movement.

In cases where vital movement is questionable, remove the cover-glass and place a drop of formalin or chloroform in the bottom of the cell; replace the cover-glass, examine and note change in character of movement, if any.

EXERCISE XX. TEST-TUBE CULTURES ILLUSTRATING FORM TYPES.

a. Make test-tube cultures in bouillon, gelatin, agar and potato of the following organisms:

Micrococcus (any species).
Sarcina lutea SCHROETER.
Pseudomonas fluorescens (FLUEGGE) MIG.
Bacillus mycoides FLUEGGE.
Microspira Metschnikovi MIG. (or any vibrio).
Spirillum rubrum v. ESMARCH.

b. Incubate all cultures, except gelatin, at 28° C.

EXERCISE XXI. STUDY OF TEST-TUBE CULTURES.

GENERAL DIRECTIONS. As soon as growth becomes visible a systematic and careful study of the cultures should be made. A detailed list of the points to be noted will be found in Chapter III, and should be consulted in writing up the descriptions. The summary below will, however, be found useful.

For bouillon cultures note: 1) condition of fluid, 2) character of sediment, 3) presence or absence of membrane, and 4) characteristic odor.

For solid cultures (agar and potato slopes), note: 1) Form of growth, 2) size, 3) surface elevation, 4) consistency, 5) color, 6) effect on media, and 7) ch aracteristic odor.

For gelatin stab cultures, note: 1) Effect on media, *a.* non-liquefying, i) line of puncture, ii) surface, *b.* liquefying, i) shape of liquefied area, ii) condition of fluid, iii) character of sediment, 2) characteristic odor.

The study should be continued from day to day as long as changes are noted. Make drawings wherever they will be of service in elucidating the descriptions.

REFERENCES. P. B. C. (Charts by Cheesman.)

SPECIAL DIRECTIONS. Study and write careful descriptions and make necessary drawings of all cultures made.

EXERCISE XXII. MICROSCOPICAL STUDY OF FORM TYPES.

a. Make cover-glass preparations from the agar streaks (XX) and stain with an aqueous solution of gentian violet or with Loeffler's methylen blue.

b. Examine with the oil-immersion objective, write the names of the organisms in their proper place in the table below.

		Name.	Sketch.
Coccaceae (spherical)	medium	.....	.....
	small	.....	.....
Bacteriaceae (elongated)	large	.....	.....
	small	.....	.....
Spirillaceae (spiral)	curved	.....	.....
	twisted	.....	.....

c. Make similar preparations from the gelatin and potato and note any variations in form, size, etc.

d. All these preparations are to be mounted in balsam, sketched (XXIII) and handed to instructor for inspection.

EXERCISE XXIII. DRAWING BACTERIA.

GENERAL DIRECTIONS. In drawing bacteria only a few organisms occuring in the microscopic field should be sketched, but these should be made of considerable size so that the exact outline may be indicated. Furthermore they should be drawn to scale and individuals selected to give range in form and size.

To measure microscopic objects an ocular micrometer is used, and the first step will be to determine its value. Place the ocular micrometer on the diaphragm in the ocular, use a *stage micrometer* as an object and focus. The image of the scale on the stage micrometer will appear imposed on that of the ocular micrometer. Make the lines of the two micrometers parallel and then make any two lines of the stage micrometer coincide with any two on the ocular micrometer, pulling out the draw-tube if necessary. Divide the value of the included space or spaces on the stage micrometer by the number of divisions on the ocular micrometer required to include them, and the quotient so obtained will give the valuation of the ocular micrometer in fractions of the units of measure of the stage micrometer (Gage). If result is not in terms of micron (μ) it should be converted to such, as this is the unit in micrometry.

In making drawings represent a micron by two millimeters on paper. This will give a magnification of 2,000 (×2,000).

REFERENCES. G. 100–108.

SPECIAL DIRECTIONS.

a. Determine the value of the ocular micrometer and fill out blanks in following table:

No. of Microscope.....Make		
	Ocular.....in., or.....mm.	
Objective.	Tube length.	Value of single division on scale in μ.
$\frac{2}{3}$ in. (16 mm.)		
$\frac{1}{6}$ in. (4 mm.)		
Oil-immersion.		

b. Make drawings of cover-glass preparations made in XXII in place provided in table.

EXERCISE XXIV. STUDY OF CELL GROUPING.

HANGING DROP PREPARATIONS.

a. Make hanging-drop preparations from bouillon cultures prepared above and also from those supplied.

b. Examine with oil-immersion objective and assign organisms to their proper place, as determined by cell grouping, in the following scheme:

		Name.	Sketch.
Isolated		.....	.....
Filaments	Bacilli	.....	.....
	Cocci	.....	.....
Plane surface, Tetrads		.....	.....
Masses	Regular	.....	.....
	Irregular	.....	.....
	Zoogloea	.....	.....

IMPRESSION PREPARATIONS. The exact relation of cell to cell as they develop in the colony can frequently be determined with greater accuracy by studying a "contact preparation" which is prepared as follows:

a. Melt a gelatin tube and slope it, when solid make a streak culture of *B. mycoides* FLUEGGE and when growth has taken place dip the tube in hot water to loosen gelatin which is then slipped out of the tube.

b. Lower gently a clean cover-glass over the surface. Apply a slight pressure by tapping glass. Raise cover-glass by one edge taking care that natural arrangement of adherent bacteria is not disturbed.

c. Thoroughly air dry the same, then fix and stain in the ordinary manner.

d. Examine the thinner layers noticing the arrangement of cells with reference to each other and draw a sufficient number to illustrate this relationship.

AGAR HANGING-DROP CULTURES.

a. Melt a tube of agar and cool to 43° C.

b. Sterilize a cover-glass by passing it two or three times through the flame quickly.

c. With the needle make a streak on the cover-glass about 3 mm. long of *B. subtilis*.

d. With the loop place a drop of liquid agar so as to cover up streak.

e. Seal cover-glass to hollow ground slide. Incubate and later examine and sketch.

EXERCISE XXV. STUDY OF INVOLUTION FORMS.

a. Grow *Bacillus subtilis* (EHRENB.) MIG. in bouillon and also in a solution containing 0.1% asparagin, 10% sugar, and by means of stained cover-glass preparations compare the individual organisms in each case in regard to their form and size. The degenerated or involution forms are more apparent by staining. Draw several cells illustrating a variety of involution forms.

b. Examine a culture of *Bacterium diphtheriae* (LOEFFLER) MIG. on Loeffler's blood serum. Read M. & R. 5.

EXERCISE XXVI. GELATIN PLATE CULTURES.

EXPLANATORY. Plate cultures are only possible with the liquefiable solid media, gelatin and agar. In making them the bacteria are mixed with the medium while it is in a fluid state in such quantities that the individuals are separated from each other by several millimeters when it is spread out on a horizontal surface to cool. As the medium solidifies, the organisms become fixed and their growth results in the formation of "colonies." These vary in size and appearance according to the peculiarities of the organism and the age of the culture, but are of the greatest service in the study and identification of the various species. These cultures are prepared as follows:

GENERAL DIRECTIONS. Three gelatin tubes are marked Nos. 1, 2 and 3 and melted by placing them in a water bath at a temperature of 42° C. For this purpose a small cup of water placed on a tripod can be used (Fig. 9). They are inoculated by introducing the material to be studied into tube No. 1. The quantity of this material varies. The amount clinging to the platinum needle will be sufficient if a pure culture is used, while in other cases several loops or even drops are necessary. The inoculated material is thoroughly mixed with the gelatin in No. 1. This is done by rolling the tube gently between the palms of the hands, instead of shaking, so as to prevent the introduction of air bubbles. With a sterile loop three loopfuls of fluid gelatin are now transferred from No. 1 to No. 2, and mixed. For method of handling tubes see Fig. 7. In like manner three or more loops from No. 2 are carried over to No. 3, which in turn is well mixed. The contents of the tubes Nos. 1–3 are now poured into separate sterile Petri dishes. The process of pouring is performed as follows: The Petri dish is placed on the desk; the gelatin tube is taken in the right hand, the cotton plug removed with the left hand; the mouth of the tube sterilized by flaming it once or twice, and when the glass is cool the gelatin is poured into the lower half of the dish while the cover is slightly raised (Fig. 10), but not inverted or laid on the table. The cover of the dish is then replaced, the test-tube filled with a solution of corrosive sublimate, and the cotton plug returned. The gelatin is spread over the entire bottom of the dish by tipping it from side to side. It is then allowed to harden by placing the dish on the cooling apparatus or leaving it on horizontal surface at room temperature. A simple, inexpensive and effective cooling apparatus is a piece of soapstone, such as is sold at hardware stores (Fig. 11). In winter this can be cooled by hanging it out of doors, at other seasons by immersing it in cold water. The three Petri dishes thus prepared should be properly labeled and placed under conditions where the gelatin will remain solid and yet growth takes place. The temperature of the laboratory should not be allowed to exceed 23° C. or gelatin cultures are in danger of melting while under examination. Within a few days colonies will make their appearance, in varying numbers, depending upon the dilution used.

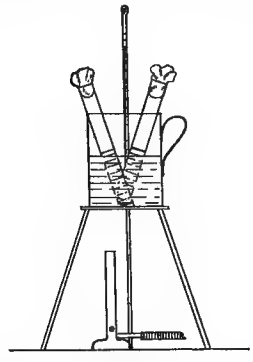


FIG. 9. Method of melting gelatin.

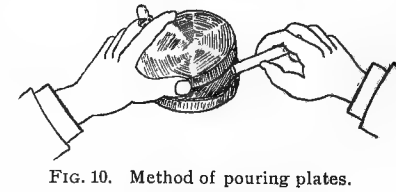


FIG. 10. Method of pouring plates.

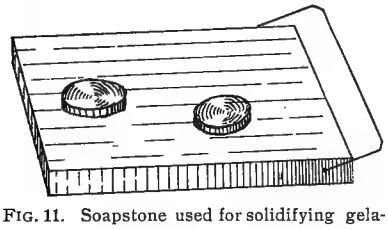


FIG. 11. Soapstone used for solidifying gelatin in Petri dishes.

Inasmuch as the first plate is invariably too thickly seeded to be of much service, this gelatin tube is often replaced by a water blank, which is treated exactly as the gelatin tube No. 1, but is not of course "plated" but simply serves to dilute the material.

REFERENCES. A. 124; H. 57; L. & K. 88; M. & W. 108; M. & R. 61; McF. 140; N. 171; P. 224; S. 72.

SPECIAL DIRECTIONS.

a. Make three gelatin plate cultures, as directed above, and inoculate with *B. subtilis*, introducing a minute portion of agar culture (XIII) into tube No. 1, two loops of No. 1 into No. 2, and three of No. 2 into No. 3. Label, and when the gelatin has solidified, place plates in cool chamber (XV).

b. Also make a "blank" plate from an uninoculated gelatin tube, observing all precautions to prevent contamination. This will serve as a control or check on your other plates. If any colonies develop on this it indicates carelessness.

EXERCISE XXVII. AGAR PLATE CULTURES.

GENERAL DIRECTIONS. These are made in the same way as the gelatin plates except that the high melting point (96° C.) of agar makes it necessary to use boiling water to melt it. Inasmuch as the vitality of vegetative bacteria is destroyed at a temperature much above 42° C., it must be cooled down before inoculating, but as agar solidifies at 39–40° C. it must not, therefore, be cooled below that point. It is best to keep the melted agar at about 42° C. for 10 minutes before it is inoculated. For this purpose a water-bath should be so arranged that the temperature can be controlled by means of a thermo-regulator. A cheap and yet satisfactory arrangement is represented in Fig. 11. Inoculate, make dilutions and pour as in case of gelatin, except that before the agar is poured, it is well to slightly warm the Petri dishes by placing them in the incubator at 38° C. for a few minutes, otherwise the agar may solidify in lumps in the plate. In cooling, agar shrinks somewhat, and in doing so water is expressed from the solid jelly. In the incubator this condenses on the under side of the cover of the Petri dish to such an extent that drops run down on to the culture surface thus causing the developing superficial colonies to "run." To obviate this the Petri dishes, when placed in the incubator, should be inverted.

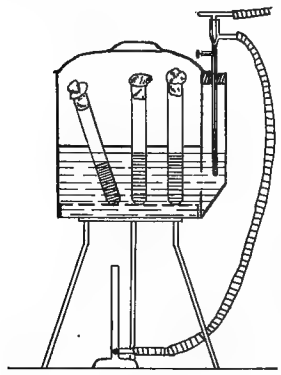


FIG. 11. Water-bath for cooling agar.

REFERENCES. H. 61; L. & K. 94; M. & R. 66; N. 285; P. 225; P. B. C. 28.

SPECIAL DIRECTIONS. *a.* Make three agar plates of *B. coli;* use one loop of bouillon culture (XIII) for tube No. 1 and proceed as in XXVI. *b.* Place in incubator at 28° C. *inverted.*

EXERCISE XXVIII. ROLL-CULTURES (Esmarch).

GENERAL DIRECTIONS. These are essentially plate cultures in which the medium instead of being poured out into dishes is solidified in a thin, even layer on the inner surface of the test-tubes. This is best accomplished by means of a piece of ice placed in a dish on a piece of cloth by which it can be kept in the desired position (Fig. 12). A horizontal groove is melted in the ice by means of a test-tube filled with hot water. In this groove the test-tubes, inoculated as in case of plate cultures, are rapidly whirled until the medium is thoroughly set. Both agar and gelatin can be used, although gelatin cannot be used suc-

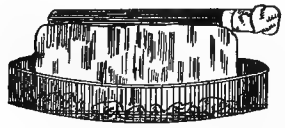


FIG. 12. Method of Making Roll-Cultures.

cessfully with those species which liquefy this medium. In the case of agar the tubes should be placed in a horizontal position a few hours (over night) until the medium has become attached to the tube; afterwards they can be stored in the usual receptacles for tube cultures.

REFERENCES. A. 131; M. & R. 65; McF. 143.

SPECIAL DIRECTIONS. *a.* Melt a tube of gelatin and without inoculating it practice making a roll-culture as described above. Avoid tipping the tube enough to get medium on cotton plug. Remelt and roll again until the knack is acquired.

b. Make two roll-cultures in gelatin of *B. coli* (XIII), using a water-blank instead of gelatin tube No. 1.

c. Make two agar cultures of *B. subtilis* in same way.

d. Incubate *b.* in cool chamber, and *c.* at 28° C.

EXERCISE XXIX. STUDY OF PLATE CULTURES.

MACROSCOPIC. As the colonies appear, note: *a.* form, *b.* size, *c.* surface elevation, *d.* consistency, *e.* color. Both the surface and deep colonies should be described as they are frequently very different. Drawings should always be made wherever they will be of value; study should be continued as long as changes are noticed. (See Chapter III, I. A. *a.–e.*)

MICROSCOPIC. The colonies appearing on the plates are to be studied under a low power of the microscope. Use a $\frac{2}{3}$ in. (16 mm.) objective. The Petri dishes can be inverted, and thus avoid the danger of exposing the culture to contamination from the air except with gelatin where liquefying organisms are present. Observe, *a.* structure of colony as a whole; *b.* character of margin. (See Chapter III. I. A. *f & g.*)

REFERENCES. P. B. C. (Cheesman's Charts.)

SPECIAL DIRECTIONS. Study, write descriptions and make drawings of all plate cultures. Use blank pages for description and sketch of cultures.

EXERCISE XXX. USE OF DECOLORIZING AGENTS.

Make three cover-glass preparations from a 24 hour old culture of *B. subtilis*, staining them with an aqueous solution of gentian violet. Mount in water and examine. While they are still under the microscope, place at one side of the cover-glass a few drops of one of the following solutions, and by means of a strip of filter paper at the opposite side draw the liquid under the cover-glass until all the color is removed. In this way determine the relative value of alcohol (95%), acetic acid (5%), and nitric acid (30%) as decoloring agents.

EXERCISE XXXI. GRAM'S STAIN.

EXPLANATORY. This is a differential stain and one of the most useful. Some bacteria when stained by this method exhibit a dark violet color, others remain perfectly colorless, thus rendering possible the differentiation of bacteria which are morphologically nearly or quite identical, and also greatly facilitating the demonstration of certain bacteria in animal tissue. Most of the pathogenic micrococci retain the violet stain although there are important exceptions. The bacilli and spirilla may or may not remain colored.

GENERAL DIRECTIONS.

a. Spread film.

b. Air dry and fix.

c. Stain with anilin-oil gentian violet 5 minutes.

d. Pour off stain and without washing:

e. Apply iodine solution 2 minutes (use several changes).

f. Decolorize with 96% alcohol until drippings do not stain white filter paper.

g. Wash in water and counter-stain with Bismarck brown.

h. Mount in water and examine.

i. Dry and mount in balsam.

REFERENCES. A. 162; H. 78; L. & K. 106; M. & W. 91; M. & R. 110; McF. 99; N. 287; P. 203.

SPECIAL DIRECTIONS. Stain films of young cultures of *B. coli* and *B. subtilis*. Also a film of an organism supplied.

EXERCISE XXXII. TUBERCLE STAIN (Gabbett).

EXPLANATORY. All of the differential methods of staining the tubercle bacterium depend upon the fact that this germ is very resistant towards the ordinary stains and in order to be stained at all must be treated with a dye containing a mordant and this either allowed to remain in contact with the micro-organism several hours or be applied hot. The latter method is the quicker and is usually employed, although it does not give as good results. When once stained this germ withstands the effect of decolorizing agents to such an extent that it is possible to remove the dye from all other objects on the cover-glass preparation (as in sputum) while it retains its own color. The application of a second dye, of a complementary color, readily distinguishes this germ from all others in the field. A few other bacteria have similar staining properties. (See Part II.) Red is the usual stain and blue the counter stain. Gabbett's method is one of the simplest.

GENERAL DIRECTIONS.

a. Spread film (sputum from tuberculous patient).

b. Air dry and fix.

c. Stain with hot carbol-fuchsin 2 minutes.

d. Wash in water.

e. Treat with Gabbett's solution $\frac{1}{2}$ to 1 minute.

f. Wash in water and examine.

g. Dry and mount in balsam.

REFERENCES. A. 162; M. & W. 92; McF. 214; P. 304.

SPECIAL DIRECTIONS. Stain three samples of sputa which contain varying numbers of the tubercle bacteria.

EXERCISE XXXIII. STAINING ENDOSPORES.

GENERAL DIRECTIONS.

A. Simple stain.

a. Prepare film as usual.

b. Fix by passing through flame 10 or 12 times instead of 3 times. (This prevents the vegetative portion from taking the stain).

c. Stain 2–5 minutes in hot carbol-fuchsin.

d. Mount and examine.

B. Differential stain (Hauser's method).

a. Make cover-glass preparation of a spore-bearing culture, fix and stain with hot carbol-fuchsin until spores are thoroughly colored. This must be determined by mounting in water and examining under microscope.

b. Cautiously decolorize with acetic acid, 5%, until stain is removed from the vegetative portion only. This to be determined as above.

c. Wash in water and counter-stain with methylen blue.

d. Examine. Crimson spores will be seen in blue bacilli.

REFERENCES. Other methods, see A. 164–167.

SPECIAL DIRECTIONS. Stain by each method spores in cultures of *B. subtilis* or other spore-bearing organisms.

EXERCISE XXXIV. STUDY OF ENDOSPORES.

a. Make cultures on peptoneless agar, or agar to which a few drops of calcium hydrate has been added, of the following organisms and incubate at 28° or 38° C. depending upon the organisms:

Bacillus subtilis (EHRENB.) COHN.
Bacterium anthracis (KOCH) MIG.
Bacillus amylobacter VAN TIEGHEM (or any clostridium form).
Bacillus tetani NICOLAIER (or any "drumstick" bacillus).
Pseudomonas erythrosporus (COHN) MIG.

b. When the cultures are 48 hours old mount films without staining, examine and note:

1) Form.
2) Size.
3) Color.
4) Power to refract light.
5) Relation to mother-cell.
   (1) Median or central.
   (2) Intermediate.
   (3) Terminal or polar.
   (4) Clostridium form.
   (5) Drumstick form.

c. Make drawings.

READ: J. H. 26; L. 60; L. & N. 76; M. & R. 6; N. 46; P. 46; P. B. C. 15; S. 114.

EXERCISE XXXV. FLAGELLA STAIN (Bunge).

GENERAL DIRECTIONS.

a. Make an agar streak of the organism to be stained.

b. After 18 to 24 hours, by means of the platinum *needle*, remove a portion of the growth (being careful to avoid the culture medium) to a large drop of *tap* water on a perfectly clean cover-glass (XV.) and allow to stand 5 minutes rather than spread, as there is less danger of breaking off the flagella.

c. Spread carefully 2 or 3 loopfuls of this drop on each of several clean cover-glasses and dry at room temperature.

d. Fix by passing the cover-glass through the top of the flame while it is held in the hand, not in the forceps, as over heating will injure the preparation.

e. Flood the cover-glasses thus prepared with the following solution (Mordant): Liquor *ferri sesquichloridi* diluted with distilled water 1:20, 1 part; saturated aqueous solution of tannic acid, 3 parts. This mixture improves with age but should be filtered before using. Allow to act 1 minute.

f. Wash in water and dry between filter paper.

g. Stain with hot carbol-fuchsin for about one minute.

h. Wash in water, dry and mount in balsam.

References. M. & W. 103; McF. 104; P. 205. Other methods M. & R. 115; McF. 101; A. 167.

Special Directions. Stain *B. typhi* from cultures furnished, also try *B. coli* and *B. subtilis.*

EXERCISE XXXVI. CAPSULE STAIN (Welch).

General Directions.

a. Spread film without the use of water.

b. Air dry.

c. Fix.

d. Apply glacial acetic acid, and drain it off immediately. *Do not wash in water.*

e. Stain with anilin-oil gentian violet (Ehrlich) which is to be renewed several times to remove acid.

f. Wash in 1 to 2% salt solution.

g. Examine in salt solution. (Balsam causes capsule to shrink.)

References. A. 163; P. 203; P. B. C. 13.

Special Directions. Use pneumonic ("rusty") sputum, or blood of rabbit infected with the pneumococcus.

CHAPTER II.

PHYSIOLOGY OF BACTERIA.

EXERCISE XXXVII. PREPARATION OF SPECIAL MEDIA.

The following media will be necessary for the work outlined in this chapter:

a. GLUCOSE BOUILLON. To ordinary bouillon add 1% glucose (C. P.), tube and sterilize in *steamer*, not in autoclave, 2 test-tubes and 2 fermentation tubes.

b. GLUCOSE GELATIN. 1% glucose (C. P.), tube and sterilize in steamer, 6 tubes.

c. GLUCOSE AGAR. 1% glucose (C. P.), " " " 5 tubes.

d. LACTOSE AGAR. 1% lactose (C. P.), " " " 2 tubes.

e. LITMUS SOLUTION. To 10 gms. of the dried material add 500 cc. of distilled water, digest in a warm place, decant clear liquid and add a few drops of nitric acid to produce a violet color. (Sutton). Place in flasks or test-tubes and sterilize in steamer three times, 1 tube.

f. DUNHAM'S SOLUTION.

Sodium chloride	0.5 gm.	Boil until all is dissolved, filter, tube and sterilize, 4 tubes.
Peptone (Witte)	1. gms.	
Water	100. "	

g. NITRATE SOLUTION.

Sodium chloride	0.5 gm.	
Peptone (Merk)	1 gms.	Filter, tube and sterilize, 3 tubes.
Potassium nitrate	0.2 "	
Water	1,000 "	

h. LITMUS MILK.

1) Freshly separated milk (or if this is not available, new milk is placed in a separatory funnel in an ice chest over night to allow the separation of the cream and milk then drawn off) is titrated with $\frac{N}{20}$NaOH and rendered slightly alkaline to phenolphthalein by the addition of $\frac{N}{1}$NaOH.

2) Litmus solution is then added until medium is faintly blue.

3) Tube and sterilize in the steamer for 30–45 minutes on 3 or 4 consecutive days. During the summer months particularly very resistant bacterial forms abound in the milk so that it is necessary to increase the number of applications or length of exposure. The efficiency of the sterilizing process should be tested by placing the flasks in the incubator for several days to see if any change occurs, 2 tubes.

In addition to the above have 15 tubes of bouillon (9 to contain exactly 10 cc. XLI & XLIV), 10 tubes of gelatin, 15 tubes of agar, 6 water-blanks and 5 potato tubes.

EXERCISE XXXVIII. EFFECT OF REACTION OF MEDIA ON GROWTH.

GENERAL DIRECTIONS.

a. Melt 6 tubes of gelatin and add, under aseptic precautions, to three of them, respectively, 0.1 cc., 0.3 cc., and 0.5 cc. of a *normal* solution of hydrochloric acid, and to the other three the same amounts of a *normal* sodium hydrate.

b. Thoroughly mix, solidify gelatin in ice water and then inoculate (stab) each tube with the organism to be studied, making a control culture in a tube of neutral gelatin.

c. Incubate at 18° C. and note the effect of the chemicals on the rate, amount and character of the growth.

REFERENCES. L. & N. 87; McF. 46.

SPECIAL DIRECTIONS. Use *B. subtilis* and *B. coli.*

EXERCISE XXXIX. EFFECT OF CONCENTRATION OF MEDIA ON GROWTH.

a. Pour about 2 cc. of "condensed milk" into each of two sterile test-tubes, dilute one with five times the volume of sterile water.

b. Inoculate both with a pure culture of *B. subtilis* and incubate at 28° C. Explain changes which occur.

c. Test extract of beef or syrup in the same way.

EXERCISE XL. EFFECT OF TEMPERATURE VARIATIONS ON RATE OF GROWTH.

GENERAL DIRECTIONS.

a. Make four agar streak cultures of organism to be studied.

b. Incubate them at the following temperatures: Ice chest (7° C.), room (20° C.), low incubator (28° C.), blood heat (38° C.).

c. By frequent observations as to luxuriance of growth, determine the optimum temperature of growth for *each.*

REFERENCES. F. 73; L. & N. 98.

SPECIAL DIRECTIONS. Use *B. campestris* and *B. coli.*

EXERCISE XLI. DETERMINATION OF THERMAL DEATH POINT.

GENERAL DIRECTIONS.

a. Make a bouillon culture of the organism to be tested.

b. 48 hours later heat a large water-bath to 45° C. Place in this, in close proximity to a thermometer, a test-tube (16 mm. in diam.) containing exactly 10 cc. of standard bouillon. (Reaction +1.5.)

c. After 15 minutes exposure at this temperature remove the cotton plug from the tube, inoculate the broth with three loopfuls (standard size, XII) of the culture prepared above (*a.*), and carefully mix by slightly agitating the tube, without removing it from the bath.

d. After a further exposure of 10 minutes remove the tube from the bath and place it in a vessel of ice cold water to cool. Then incubate at a temperature favorable to the development of the organism under observation.

e. In the same manner expose the organism to the following temperatures: 50°, 55°, 60°, and 65° C.

f. In all cases incubate at least a week and take as the thermal death point the lowest temperature at which growth fails to appear. (In more accurate work the temperature should be determined within 2° C.).

REFERENCES. P. B. C. 32.

SPECIAL DIRECTIONS. Use *B. coli* or *B. typhosus.*

EXERCISE XLII. COMPARATIVE EFFICIENCY OF DRY AND MOIST HEAT.

GENERAL DIRECTIONS.

a. Charge a water blank with culture of a spore-bearing bacillus, shaking it well to break up the clumps.

b. Sterilize eight cover-glasses by passing them several times through the flame, and place four in each of two sterile Petri dishes.

c. With a sterile loop place an equal quantity of the bacterial suspension (*a.*) on each cover-glass, and dry by placing Petri dishes in the incubator with the covers slightly raised.

d. When dry place one Petri dish in the dry sterilizer (near the thermometer), and the other in the steamer.

e. Keep both sterilizers at a temperature of 100° C., and at the end of 5, 10, 20 and 40 minutes respectively, remove one cover-glass from each Petri, place it in a sterile Petri dish and pour a tube of liquefied gelatin or agar over it. Tip the dish from side to side to dislodge as many of the bacteria as possible from the cover-glass, solidify the medium and incubate.

REFERENCES. L. 101; S. 146.

SPECIAL DIRECTIONS. Use an old (spore-bearing) culture of *B. subtilis*. Arrange data in the form of a table.

EXERCISE XLIII. EFFECT OF DESICCATION.

GENERAL DIRECTIONS.

a. Prepare five cover-glasses each of a spore-bearing and a non-spore-bearing culture, as directed in XLII.

b. Place them in a sterile Petri dish, and dry in the incubator.

c. Next morning and every twenty-four hours later plate one of the cover-glasses.

d. In this way determine the length of time the organism in question can withstand desiccation.

REFERENCES. F. 77; L. & N. 93; McF. 46; S. 151.

SPECIAL DIRECTIONS. Use a young culture of *B. coli* and an old (spore-bearing) culture of *B. subtilis*. Tabulate results.

EXERCISE XLIV. EFFECT OF CHEMICALS ON BACTERIA.

GENERAL DIRECTIONS.

a. Inoculate three tubes containing 10 cc. of sterile bouillon, with three loopfuls of a 24-hour old broth culture of organism to be studied.

b. Add 0.1 cc. of a 5% solution of carbolic acid to one tube (No. 1); 0.6 cc. to another (No. 2); and 2 cc. to the third (No. 3).

c. Two hours later transfer three loopfuls from each tube to sterile bouillon and incubate all of the tubes at 38° C.

d. The carbolic acid in No. 1 and its sub-culture does not prevent growth. In No. 2 no growth, but abundant in its sub-culture (acts as an antiseptic). In both No. 3 and its sub-culture no growth (acts as a disinfectant).

REFERENCES. F. 81; L. & N. 90; L. 107; McF. 46.

SPECIAL DIRECTIONS. Use *B. coli*.

EXERCISE XLV. RELATION TO OXYGEN.

GENERAL DIRECTIONS.

a. Pour a tube of melted agar into a sterile Petri dish, and when the medium has hardened make several parallel streaks with a platinum loop charged with an aerobic organism.

b. Sterilize a piece of mica or a cover-glass, by passing it several times through the flame and place this over several of the streaks. This is to shut out the air and should therefore be in perfect contact with the medium.

c. Make another plate in the same way using an anaerobe.

REFERENCES. F. 60; L. & N. 95; L. 180; McF. Chap. VIII.

SPECIAL DIRECTIONS. Use *B. subtilis* and an anaerobe.

EXERCISE XLVI. EFFECT OF DIRECT SUNLIGHT.

GENERAL DIRECTIONS.

a. Make an agar plate of the organism to be studied (seeding rather thickly).

b. When agar has thoroughly set, invert the Petri and paste on under side a piece of black paper from which has been cut out a number of letters, e. g., student's initials.

c. Expose this dish, paper side up, to the direct sunlight for a number of hours (4-6).

d. Remove the paper and incubate.

REFERENCES. F. 71; L. & N. 101; L. 77; McF. 46; S. 151.

SPECIAL DIRECTIONS. Use *B. prodigiosus* (Ehrenb.) Fluegge or *B. typhosus.*

EXERCISE XLVII. DETECTION OF GAS (Shake Culture).

GENERAL DIRECTIONS.

a. Melt a tube of glucose agar (or gelatin) and inoculate with a gas-producing organism.

b. Thoroughly mix and solidify quickly by placing in ice water.

c. Incubate over night.

REFERENCES. L. & N. 153; M. & R., 85.

SPECIAL DIRECTIONS. Use *B. coli;* incubate. Make sketch.

EXERCISE XLVIII. QUANTITATIVE ANALYSIS OF GAS (Fermentation Tube).

GENERAL DIRECTIONS.

a. Inoculate the open arm of a fermentation tube with a gas-producing organism.

b. Incubate at 38° C.

c. By frequent observations determine:

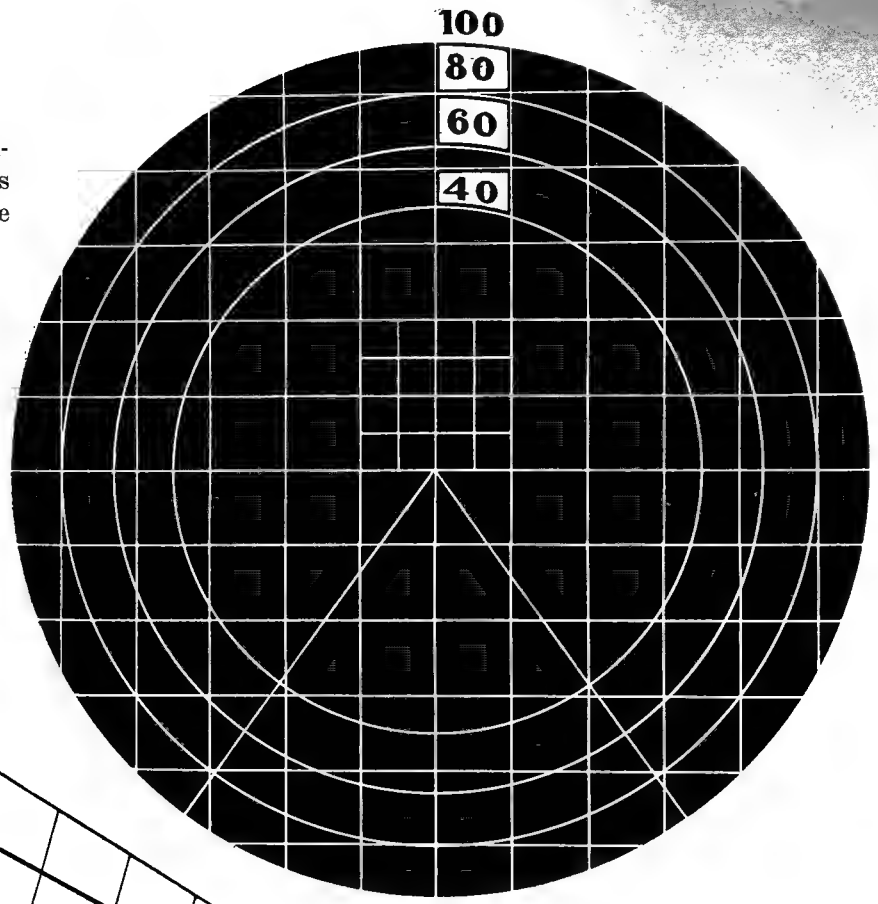
1. Whether growth takes place in the open or closed arm, i. e., whether it is aerobic or anaerobic.

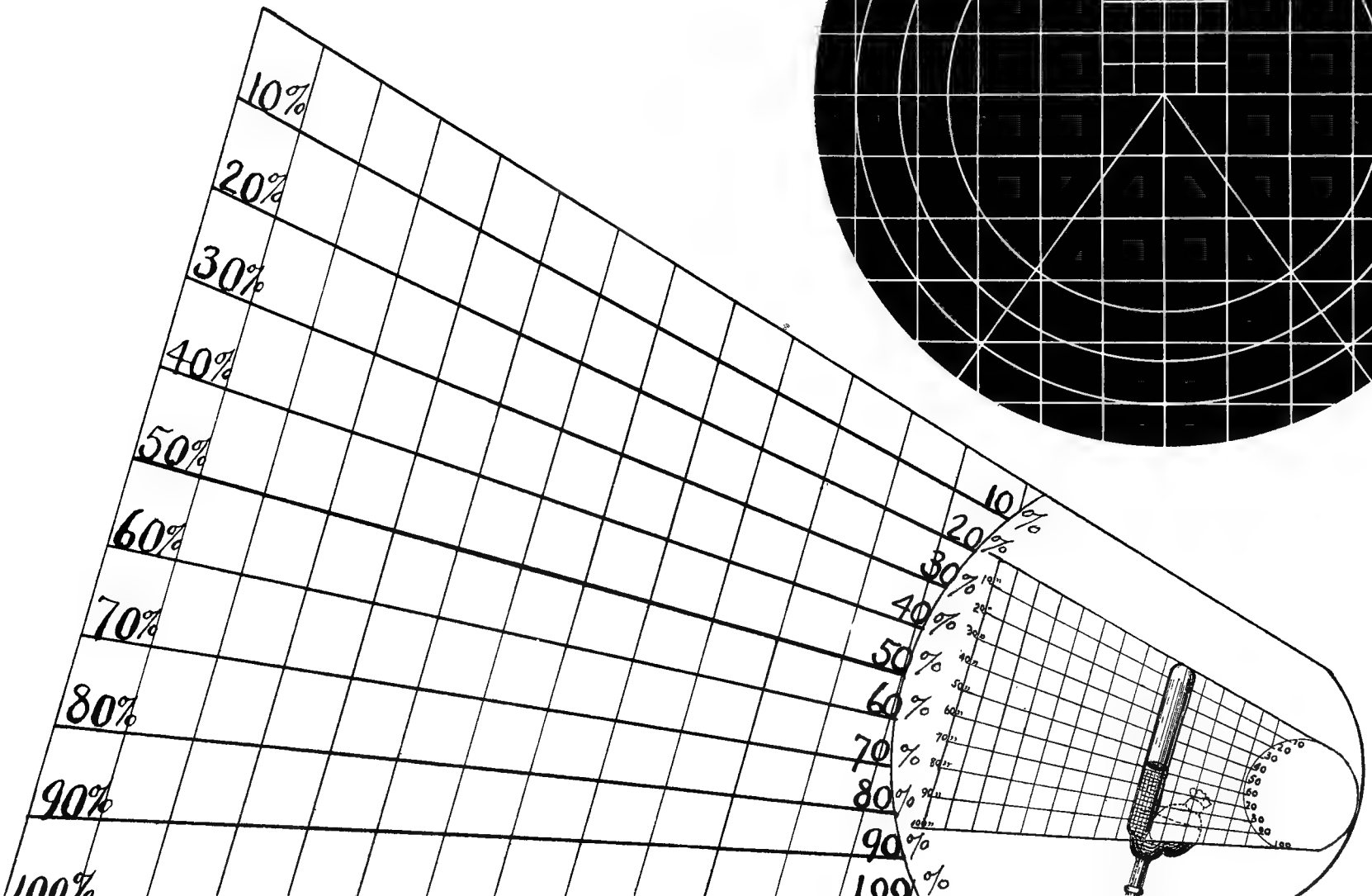
2. The rapidity and total amount of gas formation. Use gasometer. (Plate I. B.)

3. Kinds of gas. When the culture has ceased producing gas, completely fill the open arm with a 2% solution of sodium hydrate; place the thumb over the mouth of the tube and thoroughly mix the Na OH with the gas in the closed arm, then without removing the thumb return the gas to the closed arm, remove the thumb, when the medium will rise in the closed arm to take the place of the absorbed CO_2. Measure. The re-

A. PLATE COUNTER. See p. 80.

The cross-lines divide the figure into square centimeters. The numbers indicate the area of the various discs. The area of each sector is one-tenth of the whole area.





B. GASOMETER FOR FERMENTATION TUBE.

See p. 50.

maining gas is considered as hydrogen; bring this into the open arm, remove the thumb and introduce a lighted match. Air mixed with the hydrogen present causes a slight explosion. Express the amount of CO_2 and H. in the form of a proportion. $\frac{H}{CO_2} = \frac{\quad}{\quad}$.

REFERENCES. A. 203; McF. 54; M. & R. 86.

SPECIAL DIRECTIONS. Use *B. coli*, also try *B. subtilis*.

EXERCISE XLIX. DETECTION OF ACIDS (Wurtz).

GENERAL DIRECTIONS.

a. Melt a tube of lactose agar (gelatin can be used) and add enough of a sterile, blue litmus solution to give it a distinct color, cool to 42° C., inoculate it with an acid-producing organism and pour in the usual manner.

b. When the agar has solidified invert the dish and place it in the incubator.

REFERENCES. McF. 54.

SPECIAL DIRECTIONS. Use *B. coli* and incubate at 38° C.

EXERCISE L. QUANTITATIVE DETERMINATION OF ACIDS.

GENERAL DIRECTIONS.

a. Inoculate 5 test-tubes of glucose bouillon (or milk) with an acid-producing organism.

b. At periods 24 hours apart remove, with a sterile pipette, 5 cc. of the medium from each and titrate with a twentieth normal potassium (or sodium) hydrate solution, using phenolphthalien as an indicator.

c. Plot the results, expressing the number of cc. of hydrate solution as abscissae and the daily intervals as ordinates.

SPECIAL DIRECTIONS. Use *B. coli* and incubate at 38° C.

EXERCISE LI. DETECTION OF NITRITES IN CULTURES.

GENERAL DIRECTIONS.

a. Make a culture of a reducing organism in a test-tube of the nitrate solution (XXXVII. g.).

b. Incubate at 28° C. for 1 week, add 1 cc. of each of following solutions:

1) Sulphanilic acid (para-amido benzenesulphonic acid) 0.5 gm. Acetic acid (sp. gr. 1.04) 150 cc.

2) α-amido-naphthalene acetate. Boil 0.1 gram of solid α-amido-naphthalene in 20 cc. of water, filter the solution through a plug of washed absorbent cotton, and mix the filtrate with 180 cc. of diluted acetic acid. All water and vessels used must be free from nitrites. (Leffman and Beam.)

The presence of a nitrite is indicated by a pink color.

c. A tube of the original medium should be incubated and tested as a control.

REFERENCES. A. 215; McF. 56.

SPECIAL DIRECTIONS. Use *Bacillus vulgaris.* (Hauser.) Mig.

EXERCISE LII. DETECTION OF AMMONIA.

GENERAL DIRECTIONS.

a. Make bouillon culture and incubate.

b. Place in neck of tube a piece of filter paper which has been dipped in Nessler's reagent (for formula see works on water analysis). A yellow to reddish brown color indicates the presence of ammonia.

REFERENCES. L. & N. 141.

SPECIAL DIRECTIONS. Use sewage to inoculate medium.

EXERCISE LIII. DETECTION OF SULPHURETTED HYDROGEN.

GENERAL DIRECTIONS.

a. Make a culture in a test-tube, or better, a flask of bouillon and incubate at 38° C.

b. Twenty-four hours later fasten in the flask, by means of the cotton plug, a strip of filter paper moistened with lead acetate.

c. The presence of sulphuretted hydrogen is indicated by change of color from brownish to blue. The color change is often slight and can be best detected by frequent observations.

REFERENCES. L. & N. 138.

SPECIAL DIRECTIONS. Use *B. coli* or sewage.

EXERCISE LIV. DETECTION OF INDOL.

GENERAL DIRECTIONS.

a. Make a culture in a tube of glucose-free broth* (or Dunham's solution).

b. 24 hours to 1 week later add a few drops of concentrated sulphuric acid and 1 cc. of sodium nitrite solution. (Sodium nitrite, 0.02 gms. Distilled water, 100 gms.)

The presence of indol is indicated by the production of a deep red color.

REFERENCES. L. & N. 142; McF. 56; M. & R. 87.

SPECIAL DIRECTIONS. Use *B. coli*.

EXERCISE LV. DETERMINATION OF CHEMICAL ENZYMES IN CULTURES.

GENERAL DIRECTIONS.

a. Make two gelatin stab cultures of a rapidly liquefying organism and incubate several days or until the gelatin has all been liquefied.

b. Pour one into a tube of gelatin to which carbolic acid ($\frac{1}{10}$ cc. of a 5% sol. per cc. of medium) has previously been added. Mark the line which separates the liquid and solid gelatin.

c. Add the other tube of liquefied gelatin to a tube of carbolized milk.

d. Make control cultures in the carbolic media with a pure culture of the organism used above to show that the acid inhibits the growth and that the changes are not due to the living organism.

REFERENCES. McF. 53.

SPECIAL DIRECTIONS. Use *B. subtilis*.

EXERCISE LVI. VARIATION IN ENZYME PRODUCTION.

Make stab cultures of *Pseudomonas aeruginosa* (SCHROETER) MIG. (*B. pyocyaneus*), or any slow liquefier, in ordinary neutral gelatin and also glucose gelatin. Compare rate of liquefaction in each.

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*This is prepared from beef by inoculating the meat infusion with an organism capable of fermenting sugar, such as *B. coli*, and allowing it to stand several hours at 38° C. The meat is then strained and the bouillon prepared in the usual manner. This is recommended for testing for indol.

EXERCISE LVII. VARIATION IN COLOR PRODUCTION.

Make an agar streak of *B. prodigiosus*. Incubate at 38° C. 24 hours later transfer to fresh media. Continue the process of daily transplanting from cultures of previous day until chromogenic property is lost, even at the room temperature.

CHAPTER III.

TAXONOMY.

POINTS TO BE OBSERVED IN THE STUDY OF BACTERIA.

The following scheme gives an idea of the points to be noted in the description of an organism together with some of the more common descriptive terms.

CULTURE CHARACTERS.

1. GELATIN PLATE:

A. Surface colonies.

a. Form: *Punctiform*, too small to be defined by naked eye; *circular; oval; irregular; fusiform; cochlate*, twisted like a snail shell; *amoeboid*, very irregular like changing forms of amoebae; *conglomerate*, an aggregation of colonies.

b. Size, expressed in millimeters.

c. Surface Elevation: *flat; spreading; thin; raised*, growth thick with abrupt, terraced edges; *convex*, surface segment of a circle but very flatly convex; *pulvinate*, surface the segment of a circle but decidedly convex; *capitate*, hemispherical; *rough*, irregular elevations and depressions; *contoured*, like the undulating surface of a relief map; *papillate*, horn like projections; *rugose*, wrinkled; *alveolate*, depressions separated by thin walls; *pitted; sulcate*, ridged or furrowed.

d. Consistency: *thin; membraneous*, thin, dry, separating from medium; *coriaceous*, thick like leather or parchment; *viscous*, ropy; *slimy; gelatinous; brittle*.

e. Color: *transparent; vitreous*, transparent and colorless; *oleaginous*, transparent and yellow, olive to linseed oil colored; *resinous*, transparent and brown, varnish or resin colored; *translucent; paraffinous*, translucent and white, porcelaneous; *opalescent*, translucent, grayish-white by reflected light, smoky-brown by transmitted light; *nacreous*, translucent, grayish-white with pearly lustre; *sebaceous*, translucent, yellowish or grayish-white, tallowy; *butyrous*, translucent or yellow; *ceraceous*, translucent and wax colored; *opaque; cretaceous*, opaque and white; *chalky*, dull without lustre; *glossy*, shining; *fluorescent; iridescent*.

f. Margin (To be determined by low power of microscope): *entire; undulate; repand; erose*, finely eroded as if gnawed; *lobed; articulate; laciniate*, cut jaggedly into deep narrow lobes; *lacerate*, cut variously into irregular segments; *fimbricate*, edge bordered by slender processes thicker than hairs; *ciliate*, tufted; *floccose*, wooly, filaments in fleecy masses; *curled*, filaments in locks or ringlets: *filamentous*, consisting of loosely placed, interwoven filaments, not so dense as floccose.

g. Internal structure (To be determined by microscope): *homogeneous*, uniform throughout; *concentrically zoned; marmorated*, traversed by veins as in some kinds of marble, marbled; *finely punctate; areolate*, marked out with small spaces, reticulate; *moruloid*, having the character of a morula, resembling a mulberry; *segmented; finely granular; coarsely granular; grained*, as in lumber; *curled*, composed of twisted bundles of parallel filaments as in locks or ringlets; *floccose; filamentous*.

h. Change in Medium: *consistency; color; odor*.

B. Deep colonies:
- *a.* Form.
- *b.* Size.
- *c.* Color.
- *d.* Internal structure.

2. AGAR PLATES:
   - A. Surface colonies. } Same points as in gelatin plate, (1).
   - B. Deep Colonies. }

3. GELATIN STAB CULTURES.
   - A. Non-liquefying.

a. Line of puncture: *filiform*, uniform growth without any special characters; *tuberculate; papillate*, covered with papillæ; *echinulate*, minutely prickly; *villous*, beset with long or short undivided hair-like extensions; *arborescent*, beset with branched hair-like extensions; *beaded*, composed of small round more or less conjointed colonies; *banded longitudinally*.

b. Surface: (Same as surface colonies gelatin plates 1 *c.*)

B. Liquefying.

a. Shape of liquefied area: *crateriform*, saucer shaped liquefaction of gelatin; *saccate*, shape of an elongated sack, tubular; *cylindrical; funnel formed; napiform*, outline of a turnip; *fusiform*, outline of a parsnip; *stratiform*, liquefaction extending to the walls of the tube and then downward horizontally.

- *b.* Fluid: *clear; turbid; flocculent.*
- *c.* Sediment: *flocculent; stringy; granular.*
- *d.* Membrane: *character; color.*

4. STREAK CULTURES:
   - *a.* Form. }
   - *b.* Size. }
   - *c.* Surface elevation. }
   - *d.* Consistency. } Same as for colonies on gelatin plates (1).
   - *e.* Color. }
   - *f.* Margin. }
   - *g.* Internal structure. }
   - *h.* Change in medium. }

5. POTATO.
   - A. Growth apparent. (Same as plate cultures).
   - B. Growth not apparent.

6. BOUILLON:
   - *a.* Character of fluid: *clear; turbid;* etc.
   - *b.* Sediment.
   - *c.* Membrane.

7. MILK.
   - A. No visible change, even after boiling.
   - B. Curd formed:
     - *a.* Time required.
     - *b.* Character of curd: *hard; soft.*
     - *c.* Digestion.

d. Character of whey: *clear; turbid; flocculent.*

e. Reaction.

f. Gas.

g. Odor.

8. BLOOD SERUM: (Same as streak cultures).

MORPHOLOGICAL CHARACTERS.

a. Form.

b. Cell grouping.

c. Size.

1. In terms of the micromillimeter; breadth, average and extreme length.
2. In terms of human blood cell.

d. Stain.

1. Aqueous solutions; stains easily or with difficulty; uniformly or irregularly.
2. Special stain; Gram; tubercle; etc.

e. Motility.

1. Brownian movement.
2. Vital movement; sluggish or active; rotary or direct; most favorable temperature; age; media; etc.
3. Flagella; stained by Loeffler, Bunge or Van Ermengem's method; distribution, monotrichal, lophotrichal or peritrichal.

f. Capsule; stained by Ziehl, Gram or Welch's method; most favorable conditions; broad or narrow; present in serum, milk or on agar streaks.

g. Spores; time required for formation; media; position in cell, center or end; effect on shape of cell, clostridium, or drumstick; germination, time, temperature; stain, Hauser or Moeller's method; temperature limits.

h. Vacuoles (plasmoloysis).

i. Crystals.

j. Involution forms.

k. Pleomorphism.

1. Effect of various media.
2. Effect of reaction of media.

PHYSIOLOGICAL CHARACTERS.

a. Effect of desiccation.

b. Relation to temperature; minimum; optimum; maximum; thermal death point.

c. Relation to oxygen; under mica plate; in hydrogen.

d. Relation to light; (Buchner's Experiment XLVI.).

e. Relation to antiseptics and disinfectants.

f. Pigment production; relation of development to oxygen; relation of development to character of medium; changes produced by alkali and acid; solubility; spectrum analysis.

g. Gas production; rate, quantity and formula produced on glucose, lactose, and saccharose media.

h. Acid and alkali production; carbohydrates present; carbohydrates absent.

i. Relation of growth to acidity and alkalinity of medium; growth in 1.5, 3 and 4 % alkali; growth in 1.5, 3, 4 and 5 % acid.

j. Reduction of nitrates; to nitrites; to ammonia.

k. Production of sulphuretted hydrogen.

l. Production of indol.

m. Enzyme production; proteolytic; diastatic.

n. Characteristic odor.

o. Pathogenesis:

1. Modes of inoculation by which its pathogenic properties are demonstrated.
2. Quantity of material required.
3. Duration of the disease and its symptoms.
4. Lesions produced and the distribution of the bacteria in the inoculated animals.
5. Which animals are susceptible and which are immune.
6. Variations in virulence and the probable causes to which they are due.
7. Detection of toxic or immunizing products of growth.
8. Widal test.
9. Pfeiffer's phenomenon.

REFERENCES: Chester, Report Delaware Experiment Station, 1897; A. 216; P. B. C. (Cheesman's Charts).

CLASSIFICATION OF BACTERIA. (MIGULA.)

I. Cells globose in a free state, not elongated in any direction before divisions in 1, 2, or 3 planes. — COCCACEAE ZOPH emend. MIG.

A. Cells without organs of motion.

a. Division in one plane, - - 1. *Streptococcus* BILLROTH.

b. Division in two planes, - - 2. *Micrococcus* (HALLIER) COHN.

c. Division in three planes, - - 3. *Sarcina* Goodsir.

B. Cells with organs of motion.

a. Division in two planes, - - 4. *Planococcus* MIGULA.

b. Division in three planes, - - 5. *Planosarcina* MIGULA.

II. Cells cylindrical, longer or shorter, and only divided in one plane, and elongated to twice the normal length before the division.

(1) Cells straight, rod-shaped without sheath, non-motile by means of flagella. — BACTERIACEAE MIGULA.

A. Cells without organs of motion, - 6. *Bacterium* EHRENB.

B. Cells with organs of motion (flagella).

a. Flagella distributed over the whole body, - - - - 7. *Bacillus* COHN.

b. Flagella polar, - - - 8. *Pseudomonas* MIGULA.

(2) Cells crooked, without sheath. — SPIRILLACEAE MIGULA.

A. Cells rigid, not snake-like or flexuous.

a. Cells without organs of motion (flagella), - - - - - - 9. *Spirosoma* MIGULA.
b. Cells with organs of motion (flagella)
1. Cells with 1, very rarely 2–3 polar flagella, - - - 10. *Microspira* SCHROETER.
2. Cells with polar flagella-tufts, 11. *Spirillum* EHRENB.
B. Cells flexuous, - - - - - 12. *Spirochaeta* EHRENB.
(3) Cells enclosed in a sheath. CHLAMYDOBACTERIACEAE MIGULA.
A. Cell contents without granules of sulphur.
a. Cell threads unbranched.
1). Cells division always only in one plane, - - - - - 13. *Streptothrix* COHN.
2). Cell division in three planes previous to the formation of condia.
i). Cells surrounded by very delicate scarcely visible sheath (marine), - - 14. *Phragmidiothrix* ENGLER.
ii). Sheath clearly visible (fresh water), - - 15. *Crenothrix* COHN.
b. Cell threads branched, - - 16. *Cladothrix* COHN.
B. Cell contents containing sulphur granules. 17. *Thiothrix* WINOGRADSKY.
(4). Cells destitute of a sheath, united into threads motile by means of an undulating membrane. BEGGIATOACEAE.
Only one genus. (The single species is scarcely separable from Oscillaria) - 18. *Beggiatoa* TRAVISAN.

CHAPTER IV.

SYSTEMATIC STUDY OF REPRESENTATIVE NON-PATHOGENIC BACTERIA.

EXERCISE LVIII. PREPARATION OF SPECIAL MEDIA.

Tube and sterilize the following media for work in Chapters IV and V:

80 tubes of plain agar.
2 tubes of lactose agar.
20 tubes of gelatin.
8 tubes of bouillon.
10 fermentation tubes of glucose bouillon.
8 tubes of potato.
8 tubes of milk.
8 tubes of Dunham's solution.
10 water-blanks.

EXERCISE LIX. BACILLUS PRODIGIOSUS (Ehrenb.) Fluegge.

EXPLANATORY. This organism was first described by Ehrenberg (Erhandlunger der Berliner Akademie) in 1839 and named Monas prodigiosa. It is the oldest known chromogenic bacterium. It is commonly found in the air of Europe and has a very interesting history on account of its casual relation to bread epidemics—"bloody bread," "bleeding host," etc. It is questionable if it occurs spontaneously in this country. It is slightly pathogenic. Introduced intraperitoneally into guinea pigs in large quantities it produces death. Inoculated into animals naturally immune to malignant oedema it renders them susceptible. Rabbits inoculated with anthrax are protected by a subsequent inoculation with this organism. It is grown with the streptococcus of erysipelas to produce Coley's Fluid for treatment of inoperable malignant tumors.

REFERENCES. Lafar, 137–138.

MORPHOLOGICAL CHARACTERS.	Age of cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores:			
7. Special characters, such as:			
deposits, vacuoles,			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature :

2. Relation to free oxygen:

3. Relation to other agents, such as

desiccation, light, disinfectants, etc.:

4. Pigment production:

5. Gas production in glucose media:

a. Shake culture

b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

(3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.

(4) reaction in open arm: (5) gas formula : H : CO_2 : : :

6. Acid or alkali production, litmus milk.

7. Reduction of nitrates; to nitrites, to ammonia

8. Indol production; 24 hours, 48 hours, days

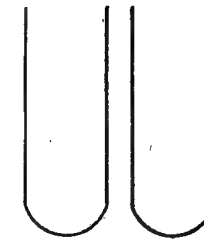
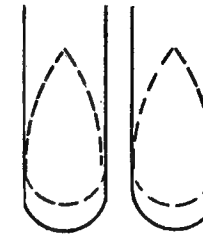
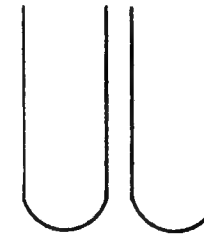
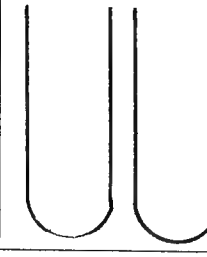
fecal odor; 24 hours, 48 hours, days

9. Enzyme production : proteolytic diastatic

10. Characteristic odor

11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) latin plate: Surface Colonies. Deep Colonies.					
(2) ar plate: Surface Colonies. Deep Colonies.					
(3) atin tab.					
(4) r reak.					
(5) to.					
(6) llon.					
(7) al dia.					

EXERCISE LX. VARIETY OF PIGMENTS.

Make agar or potato streak cultures of the following organisms, incubate at 28°C., study, describe and sketch.

Agar Streak.	24 Hours.	48 Hours.	Sketches.
Bacillus indicus or _____ _____			
Sarcina aurantiaca or _____ _____			
Sarcina lutea or _____ _____			
Pseudomonas fluorescens (B. fluorescens) or _____ _____			
Pseudomonas aeruginosa (B. pyocyaneus) or _____ _____			
Pseudomonas violacea or _____ _____			

EXERCISE LXI. SEPARATION OF BACTERIAL COLORING MATTER.

a. Make four agar streaks of Bacillus prodigiosus, which are to be kept in the dark until the coloring matter is well formed.

b. Add about 10 cc. of ether to each tube and shake vigorously until the red pigment has all been dissolved out.

c. Pour into a large test-tube and allow to stand over night in the dark, then pipette off the colored portion.

d. Divide this into four parts and treat them as follows:

1. Evaporate on glass slide and examine crystals formed under microscope.
2. Add a few drops of hydrochloric acid, drop by drop.
3. Add a few drops of sodium hydroxide.
4. Stand in direct sunlight.

EXERCISE LXII. BACTERIUM PHOSPHORESCENS Fischer.

General Considerations. Described by Fischer in 1887 (Zeitschrift für Hygiene, Band II, p. 92). Found in Kiel harbor, dead sea fish, oysters and occasionally on meat in shops. The production of light is shown in the dark, especially when the organism is grown on a medium made by boiling two salt herrings in a liter of water, adding 100 gms. of gelatin to the filtrate without neutralization, tubing and then sterilizing (Lehmann). Phosphorescence can even be restored to attenuated cultures by growth on this medium. Inasmuch as oxygen is necessary to light production surface growths are best.

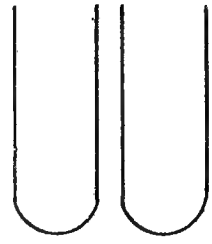
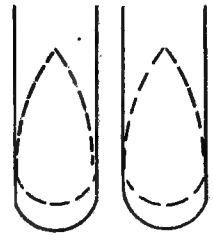
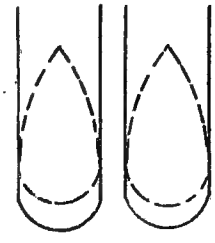
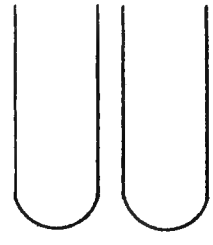
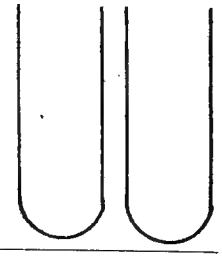
References. Lafar 160-164.

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

Physiological Characters.

1. Relation to temperature :
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc.:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.
   (4) reaction in open arm: (5) gas formula, H : CO_2 : :
6. Acid or alkali production, litmus milk.
7. Reduction of nitrates; to nitrites, to ammonia
8. Indol production; 24 hours, 48 hours, days
   fecal odor; 24 hours, 48 hours, days
9. Enzyme production : proteolytic diastatic
10. Characteristic odor
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXIII. BACILLUS ACIDI LACTICI Hueppe.

General Considerations. First described in 1884 by Hueppe in Mitteil. aus dem Kaiserl. Gesundheitsamte, Bd. II, p. 1837. This organism may be taken as a type of the bacteria causing sour milk.

References. Lafar, 222–244.

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....
3. Relation to other agents, such as: .....
   desiccation, light, disinfectants, etc.: .....
4. Pigment production: .....

.....

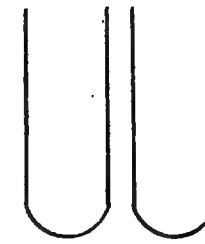
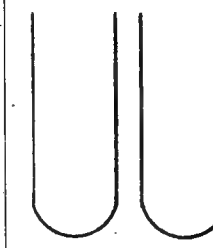
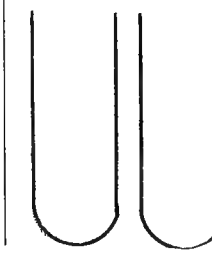
5. Gas production in glucose media:
   a. Shake culture .....
   b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....
   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.
   (4) reaction in open arm: ..... (5) gas formula, $H : CO_2$: : :
6. Acid or alkali production, litmus milk. .....
7. Reduction of nitrates; to nitrites ....., to ammonia .....
8. Indol production; 24 hours ....., 48 hours ..... days .....
   fecal odor; 24 hours ....., 48 hours ..... days .....
9. Enzyme production: proteolytic ..... diastatic .....

.....

.....

10. Characteristic odor .....
11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXIV. BACILLUS VULGARIS (Hauser) Migula.

PROTEUS VULGARIS.

GENERAL CONSIDERATIONS. Described by Hauser in 1885 as Proteus vulgaris (Ueber Faulnis Bakterien). It is widely distributed and is commonly found in putrefactive substances. It is one of several related species included under the old name of "Bacterium termo." While in small doses and under ordinary conditions it is harmless, at times and in large doses it may be pathogenic.

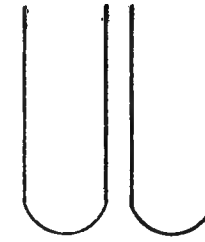
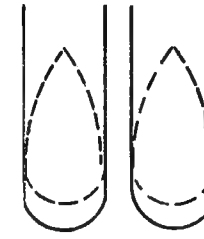
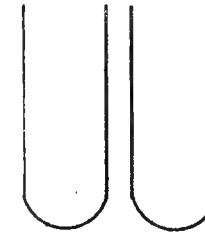
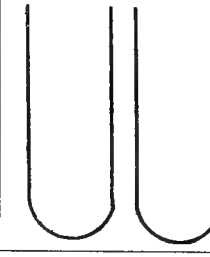
REFERENCES. Lafar 194–199.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.
   (4) reaction in open arm: (5) gas formula, H : CO_2 : :
6. Acid or alkali production, litmus milk
7. Reduction of nitrates; to nitrites, to ammonia
8. Indol production; 24 hours, 48 hours days
   fecal odor; 24 hours, 48 hours days
9. Enzyme production: proteolytic diastatic
10. Characteristic odor
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Name of organism .....

Source, habitat, etc. .....

.....

References .....

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon	.....	.....	
.....	.....	.....	
b. Agar	.....	.....	
.....	.....	.....	
c. Gelatin	.....	.....	
.....	.....	.....	
d. Other media	.....	.....	
2. Size	.....	.....	
3. Cell groupings	.....	.....	
and arrangements	.....	.....	
in growths	.....	.....	
4. Staining powers:	.....	.....	
a. Aqueous gentian-violet	.....	.....	
b. Loeffler's methylen-blue	.....	.....	
c. Gram's stain	.....	.....	
d. Special stains	.....	.....	
5. Motility:	.....	.....	
a. Character of movement	.....	.....	
b. Flagella stain	.....	.....	
6. Spores	.....	.....	
.....	.....	.....	
7. Special characters, such as:	.....	.....	
deposits, vacuoles	.....	.....	
pleomorphic and involution forms, capsules, etc	.....	.....	

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.

(4) reaction in open arm: ..... (5) gas formula, $H : CO_2$: : :

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ...., to ammonia .....

8. Indol production; 24 hours ....., 48 hours ..... days .....

fecal odor; 24 hours ....., 48 hours ..... days .....

9. Enzyme production: proteolytic ..... diastatic .....

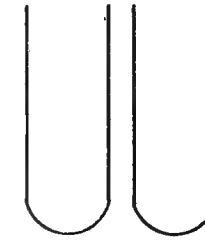
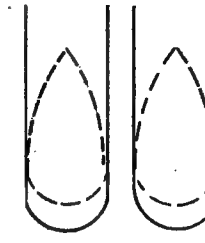
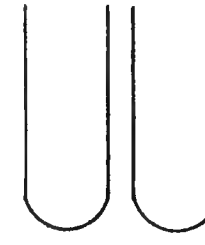
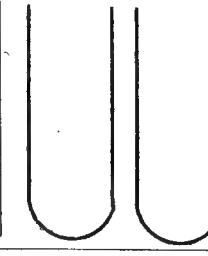
.....

.....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Name of organism ....

Source, habitat, etc. ....

References ....

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

Physiological Characters.

1. Relation to temperature :

2. Relation to free oxygen:

3. Relation to other agents, such as:

   desiccation, light, disinfectants, etc.:

4. Pigment production:

5. Gas production in glucose media:

   a. Shake culture

   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.

   (4) reaction in open arm: (5) gas formula, H : CO_2 : : :

6. Acid or alkali production, litmus milk.

7. Reduction of nitrates; to nitrites, to ammonia

8. Indol production; 24 hours ..... 48 hours, ... days

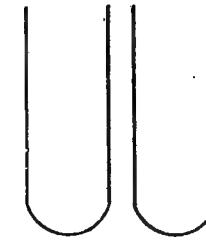
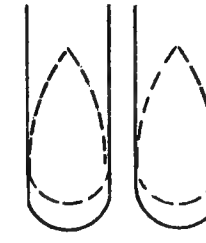
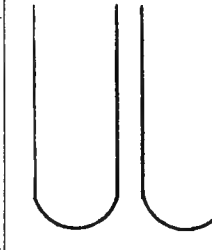
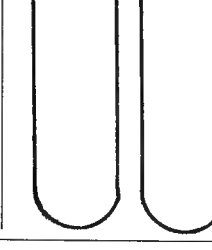
   fecal odor; 24 hours ..... 48 hours, ... days

9. Enzyme production : proteolytic ..... diastatic

10. Characteristic odor

11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Name of organism .....

Source, habitat, etc. .....

.....

References .....

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon			
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b. Agar			
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c. Gelatin			
.....			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
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5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
.....			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....
3. Relation to other agents, such as: .....
   desiccation, light, disinfectants, etc.: .....
4. Pigment production: .....

.....

5. Gas production in glucose media:
   a. Shake culture .....
   b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....
   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.
   (4) reaction in open arm: ..... (5) gas formula, $H : CO_2$: : :
6. Acid or alkali production, litmus milk. .....
7. Reduction of nitrates; to nitrites ..... , to ammonia .....
8. Indol production; 24 hours ..... , 48 hours ..... days .....
   fecal odor; 24 hours ..... , 48 hours ..... days .....
9. Enzyme production: proteolytic ..... diastatic .....

.....

.....

10. Characteristic odor .....
11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
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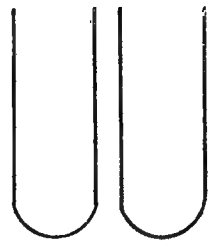
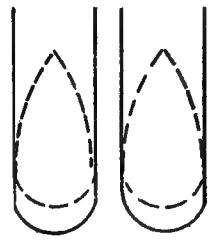
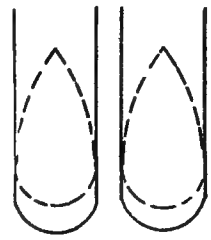
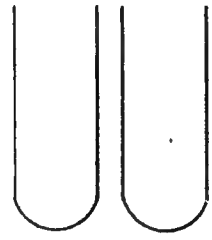
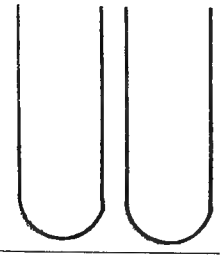
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

CHAPTER V.

BACTERIOLOGICAL ANALYSIS.

EXERCISE LXV. COMPARATIVE ANALYSIS OF AIR (Koch's Method).

a. Plate three tubes of gelatin and expose by removing lid for 20 minutes in the following places: 1. Laboratory, 2. Cellar, 3. Out of doors.

b. Replace the lids and keep plates at 22° C. for several days.

c. Count the colonies; if the number of colonies is greater than 100, use the counting plate figured in Plate I. A. and count a portion and estimate the whole number.

d. Calculate the area of the Petri dish by multiplying the square of the diameter by 0.785.

e. Express the results in terms of the number of organisms which fall per square foot per minute.

This method enables one to make a rough comparison of the number of organisms occurring in the localities examined, but to determine the number per volume the following method must be employed.

REFERENCES. H. 390.

EXERCISE LXVI. QUANTITATIVE DETERMINATION OF NUMBER OF BACTERIA IN AIR (Petri-Sedgwick Method).

GENERAL DIRECTIONS.

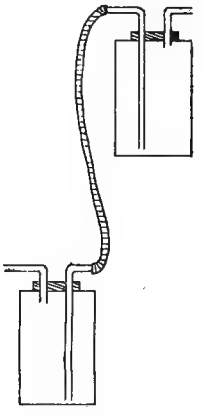


FIG. 14. Aspirator for filtering air.

a. A piece of glass tubing 6 mm. ($\frac{2}{3}$ in.) in diameter by 15 cm. (6 in.) long is drawn out at one end in a gas flame and sealed.

b. Fill this tube about one-third full with granulated sugar, insert a cotton plug next to the sugar and one at the end of the tube (Fig.13).

c. Sterilize in the hot air sterilizer for 1 and ½ hours at 130° C. (sugar melts at a higher temperature).

d. Fasten the tube, pointed end up, in a clamp, remove the first cotton plug and connect with an aspirator. (Fig. 14).

e. Break off the pointed end of the tube and draw a measured quantity of air through the sugar.

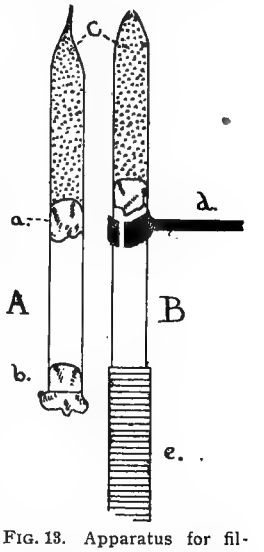


FIG. 13. Apparatus for filtering air through sugar. A, ready for sterilization. B, point broken off and attached to aspirator.

SPECIAL DIRECTIONS.

a. Filter 50 liters of air.

b. Dissolve sugar in 10 cc. of sterile water and make plates, using 1 cc. of the mixture.

c. Incubate, count colonies as above and estimate the number of organisms per liter of air.

REFERENCES. A. 551; H. 393; L. & K. 392; McF. 164; N. 449; S. 541.

EXERCISE LXVII. RELATION OF BACTERIA IN THE AIR TO DUST PARTICLES.

a. Pour a tube of gelatin into a Petri dish and solidify.

b. Remove the lid and shake a dust-brush or cloth over it.

c. 18-24 hours later, examine under low power of microscope to determine the relation of the developing colonies to the dust particles.

EXERCISE LXVIII. ESTIMATION OF NUMBER OF BACTERIA IN SOIL.

a. With a sterile knife collect a sample of soil in a sterile test-tube or Petri dish. Samples at various depths can be secured by means of an earth borer. (Fig. 15).

b. Weigh out 1 gram and dilute 1000 times with sterile water.

c. Make three gelatin plate cultures using 1 cc., $\frac{1}{2}$ cc. and $\frac{1}{10}$ cc. of this suspension. Incubate.

d. Count the colonies as they develop and estimate the number of bacteria per gram of soil.

e. Many of the bacteria of the soil are anaerobic and can only be grown in the absence of free oxygen. See Part II. Chap. VII. for methods of cultivation.

REFERENCES. A. 556; H. 394; L. & K. 389; McF. 174; N. 444; S. 567.

Fig. 15. Fraenkel's Soil Borer.

EXERCISE LXIX. WATER ANALYSIS.

QUANTITATIVE ANALYSIS.

a. Collect a sample of water in a sterile test-tube or bottle. Fig. 16 shows a form of apparatus used in taking samples of water at various depths.

b. Make two gelatin plates using $\frac{1}{2}$ cc. and $\frac{1}{10}$ cc. of the water.

c. Count the colonies as they appear, and estimate the number per cc.

d. Make agar plates and compare results with those obtained above.

e. Analyze a surface water (lake or river), a deep well and a spring water.

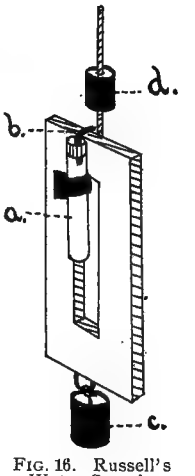


FIG. 16. Russell's Water Sampler.

QUALITATIVE ANALYSIS.

a. Detection of putrefactive organisms. Examine gelatin plates, made above and (1) determine number of liquefying organisms per cc. (2) search for the presence of proteus forms. (*B. vulgaris.*)

b. Detection of Faecal Bacteria.

1) Inoculate a fermentation-tube containing glucose bouillon (1%) with 1 cc. of water.

2) Make litmus lactose agar plate using 1 cc. water.

3) Incubate both at 38° C.

4) Compare growth obtained with that of *B. coli.*

REFERENCES. A. 526; H. 373; L. & K. 396; McF. 169; M. & R. 79; N. 422; P. 245; S. 553. For the determination of the various species present see Frankland's Micro-organisms of Water; Fuller: Report Am. Public Health Assoc., 1899, 580.

EXERCISE LXX. QUANTITATIVE ANALYSIS OF MILK.

a. Obtain a sample of milk in a sterile vessel.
b. Dilute milk 1000 times with sterile water.
c. Make plates as under soil (LXVIII).
d. Count colonies and estimate number of bacteria per cc.

EXERCISE LXXI. EFFICIENCY OF PASTEURIZATION.

a. Place same milk as used in previous experiment in the bottles of a pasteurizing apparatus, such as Freeman's, and pasteurize as per printed directions, or place the milk in ordinary milk bottles or fruit jars, filling to a uniform level; these are then to be placed in a flat bottomed pail which is to be filled with water and heated to 71° C.(160° F.), remove source of heat, cover and allow to stand 30 minutes. Remove bottles and cool as quickly as possible without danger to glass.

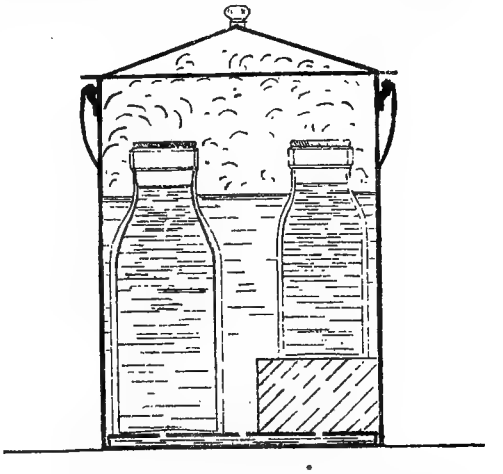


FIG. 17. A home-made pasteurizer (Russell.)

b. Determine bacterial content of pasteurized product by making plates. A dilution of 100 will probably be sufficient. Express results so as to indicate per cent. of organisms destroyed by the process. Compare the keeping qualities of the pasteurized product with that of the raw milk by keeping samples of both under similar conditions, e. g. in locker or ice chest, making frequent observations.

Pasteurized milk should not have a permanently cooked taste.

REFERENCES. Bull. Wis. Exp. Station No. 44. Russell, Outlines of Dairy Bacteriology, 95 (4th Edit.).

EXERCISE LXXII. TESTING ANTISEPTIC ACTION OF CHEMICALS.

GENERAL DIRECTIONS.

a. Fill a number of test-tubes with a measured quantity of agar (5 cc.).

b. Add to the agar varying but measured amounts of the substance to be tested. If the antiseptic is not volatile, or affected by heat, sterilize.

c. Inoculate the tubes thus prepared, together with a control, with *B. coli* and make rolls.

d. Keep these cultures under observation in the 28° C. incubator.

e. If no growth appears within 96 hours repeat the experiment, using smaller amounts of the antiseptic. In this way determine the amount of chemical (in %) which just prevents growth.

SPECIAL DIRECTIONS. Test in this way carbolic acid (5 %), alcohol (95 %).

REFERENCES. A. 566; H. 411; N. 527; S, 156.

EXERCISE LXXIII. TESTING DISINFECTING ACTION OF CHEMICALS.

SUSPENSION METHOD.

a. Make a culture of the organism to be studied in tubes of bouillon containing 5 cc.

b. Incubate at 38° C. for 24 hours.

c. Add to this an equal amount (5 cc.) of the disinfectant to be tested, of *double the required strength.*

d. At the end of 5, 10, 20, 40, and 60 minutes make agar rolls, using two or three loopfuls of the mixture for each roll.

e. In this way determine the time of exposure necessary to kill the organism used.

f. Test in this way the value of corrosive sublimate (1:1000) and Lysol (5%), using *B. coli.*

COVER-GLASS METHOD.

a. Make a bouillon culture of the organism to be studied and incubate at 38° C. for 24 hours.

b. By means of a burette, pipette, or loop, place the same sized drop on each of several sterile cover-glasses and dry as directed in the experiment on desiccation (XLIII).

c. When the cover-glasses are dry, they are to be immersed in the disinfectant for the desired time, then removed and transferred to tubes of melted agar which are then made into rolls.

d. Test by this method carbolic acid (5%), alcohol (95%) and formaldehyde (10%), using *B. coli.*

References. A. 558; N. 518; P. 152; S. 158.

PART II.

MEDICAL BACTERIOLOGY.

PART II.—MEDICAL BACTERIOLOGY.

CHAPTER VI.

PATHOGENIC AEROBES.

EXERCISE LXXIV. PREPARATION OF CULTURE MEDIA.

The following media will be necessary for the work outlined in the following chapters. This is exclusive of a few special media which are described under special heads and are to be performed as a part of the exercise in which they are used.

100 tubes of agar.
12 tubes of glucose agar.
100 tubes of gelatin.
12 tubes of glucose gelatin.
30 tubes of bouillon.
30 fermentation tubes of glucose bouillon.
30 tubes of potato.
30 tubes of milk.
30 tubes of glucose free broth or Dunham's solution.
30 water blanks.
30 tubes of blood serum:

a. Collection of the blood. Sterilize Mason fruit jars, by successive washings in corrosive sublimate, distilled water, alcohol and ether (or sterile Erlenmeyer flasks may be used). These are to be carried to the slaughter house and the blood from a beef caught directly into them. They are then allowed to stand undisturbed for 15–30 minutes, or until the clot has firmly attached itself to the sides of the vessel, when they may be removed to the laboratory.

b. Separation of the serum from the blood clot. The clot is separated from the sides of the vessel by means of a sterile knife or glass rod, and the vessel placed in the ice chest. After standing 48 hours the clot will have shrunken away from the walls of the vessel leaving the clear serum on the top and at the sides. This can now be pipetted or siphoned off. If the serum contains a large number of red blood corpuscles it can be placed in rather tall cylinders (graduates) and allowed to stand 24 hours longer, when the clear straw colored serum can be readily separated. This may be preserved for a long time by the addition of $\frac{1}{2}$ % chloroform and kept in a tightly corked bottle in a cool place.

c. Loeffler's mixture. This consists of 3 parts of blood serum and 1 part of glucose bouillon (1 %).

d. Sterilization. Fill sterile test-tubes (about 3 cm. deep) with the serum and sterilize either:

(1) By heating to 60–65° C. for 1 hour on 5 successive days, and finally placing the tubes in a sloped position in inspissator (or sloping tray in a high temperature incubator or steamer) and heated above the coagulating point of the serum (70° C.) In this method the clear serum is used and not Loeffler's mixture and a transparent medium obtained. This method is not usually employed, but the following:

(2) Loeffler's mixture is used and the tubes are immediately placed in a sloping position in an inspissator, or steamer and heated up to 95° C. for 1 hour on three consecutive days. If a higher temperature is employed bubbles are formed which rupture the surface of the medium in their escape. When sterile the tubes should be sealed with paraffin or otherwise.

References. A. 106; H. 45; L. & K. 83; M. & R. 50; M. & W. 81; McF. 131; N. 463; P. 219; S. 37 & 35.

EXERCISE LXXV. STREPTOCOCCUS PYOGENES Rosenbach.

First described by Rosenbach in 1884. It is found in abscesses, pyemia, puerperal fever, and erysipelas. It is frequently present in mixed or secondary infections and occurs in the mouth and sputum and on the mucous membranes of the nose, urethra, vagina, etc.

REFERENCES. Rosenbach: Mikroorganismen bei den Wundinfectionskrankheiten des Menschen, 1884. A. 268; H. 133; L. & K. 117; M. & R. 168; M. & W. 124; McF. 190; P. 476; S. 274.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....
   desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

5. Gas production in glucose media:
   a. Shake culture .....
   b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....
   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.
   (4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : :

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ..... to ammonia .....

8. Indol production; 24 hours ....., 48 hours ..... days .....
   fecal odor; 24 hours ....., 48 hours ..... days .....

9. Enzyme production: proteolytic ..... diastatic .....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
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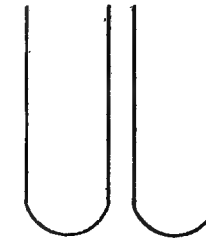
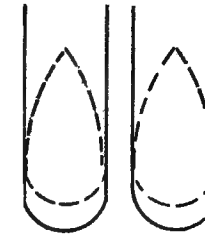
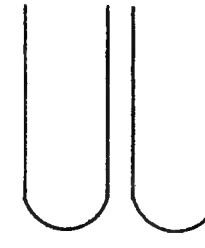
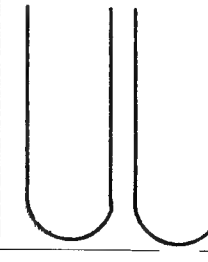
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXVI. *MICROCOCCUS PYOGENES* (Rosenbach) Mig.

STAPHYLOCOCCUS PYOGENES ALBUS; STAPHYLOCOCCUS EPIDERMIDIS ALBUS.

First described by Rosenbach, in 1884. One of the common organisms found in pus. Occurs on the skin, in sputum, air, water, dust and soil.

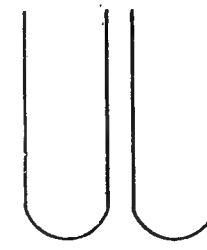
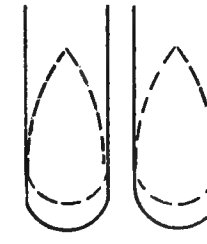
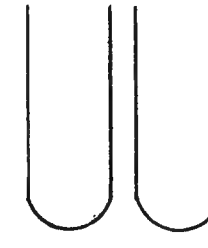
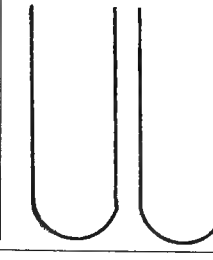
REFERENCES. Rosenbach: Mikroorganismen bei dem Wundinfectionskrankheiten des Menschen. 1884. McF. 184; P. 470; S. 272.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature :
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc.:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.
   (4) reaction in open arm: (5) gas formula, H : CO_2 : :
6. Acid or alkali production, litmus milk.
7. Reduction of nitrates; to nitrites, to ammonia
8. Indol production; 24 hours, 48 hours, days
   fecal odor; 24 hours, 48 hours, days
9. Enzyme production : proteolytic diastatic
10. Characteristic odor
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXVII. MICROCOCCUS MELTINESIS Bruce.

This organism is the cause of Malta fever and is found especially in the spleen of the diseased.

REFERENCES. Bruce: Ann. de l' Inst. Pasteur, 1899, 8; 289. Durham: Jour. Path. and Bact., 1898, 5; 377. H. 361; M. & R. 449.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc.			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:

2. Relation to free oxygen:

3. Relation to other agents, such as:

desiccation, light, disinfectants, etc.:

4. Pigment production:

5. Gas production in glucose media:

a. Shake culture

b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

(3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.

(4) reaction in open arm: (5) gas formula, H : CO_2 : : :

6. Acid or alkali production, litmus milk.

7. Reduction of nitrates; to nitrites, to ammonia.

8. Indol production; 24 hours, 48 hours days

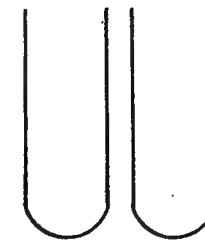
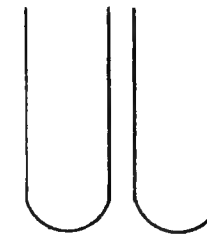
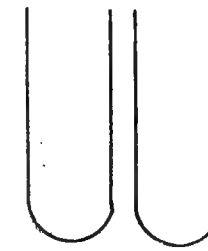
fecal odor; 24 hours, 48 hours days

9. Enzyme production: proteolytic diastatic

10. Characteristic odor.

11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) elatin plate: a) Surface Colonies. b) Deep Colonies.					
(2) Agar plate: a) Surface Colonies. b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXVIII. MICROCOCCUS AUREUS (Rosenbach) Mig.

STAPHYLOCOCCUS PYOGENES AUREUS; GOLDEN PUS COCCUS.

First described in 1884 by Rosenbach. It is the most common organism in pus.—80%.

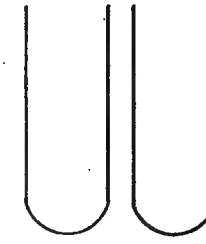
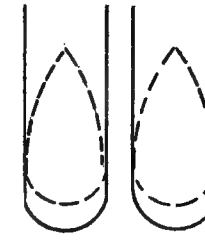
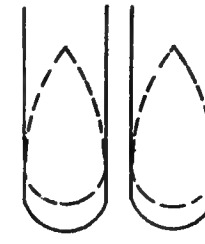
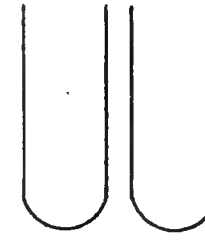
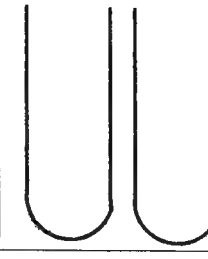
REFERENCES. Rosenbach: Mikroorganismen bei dem Wundinfektionskrankheiten des Menschen. A. 260; H. 130; L. & K. 115; M. & R. 166; M. & W. 121; McF. 184; P. 461; S. 265.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.
   (4) reaction in open arm: (5) gas formula, H : CO_2 : :
6. Acid or alkali production, litmus milk
7. Reduction of nitrates; to nitrites, to ammonia
8. Indol production; 24 hours, 48 hours days
   fecal odor; 24 hours, 48 hours days
9. Enzyme production: proteolytic diastatic
10. Characteristic odor
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXIX. MICROCOCCUS GONORRHOEÆ (Neisser) Fluegge.

GONOCOCCUS; DIPLOCOCCUS OF GONORRHOEA.

First described in 1879 by Neisser. It is constantly found in gonorrhoeal discharges and may produce disease on any mucous membrane; urethra, bladder, rectum, conjunctiva (causing ophthalmia neonatorum), and even cause arthritis (gonorrhoeal rheumatism), endocarditis, salpingitis and general septicaemia.

REFERENCES. Neisser: Cent. f. d. Mediz. Wissensch., 1879, 497; Foulerton: Trans. Brit. Inst. of Prev. Med., 1897, 1:40; A. 277; H. 145; L. & K. 311; M. & R. 189; M. & W. 130; McF. 201; P. 522; S. 283.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc.			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.

(4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : :

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ....., to ammonia .....

8. Indol production; 24 hours ..... 48 hours ..... days.....

fecal odor; 24 hours ..... 48 hours ..... days.....

9. Enzyme production: proteolytic ..... diastatic .....

.....

.....

10. Characteristic odor .....

11. Pathogenesis .....

The *Micrococcus gonorrhoeae* does not grow on the ordinary artificial media but may be cultivated on the following:

a. Blood agar. Blood drawn from the finger, under aseptic precautions, in a capillary pipette is placed on the surface of agar either in tube or Petri dish. This blood is then inoculated with the material containing the organism (pus or pure culture) and smeared over the surface of the agar either with the loop or better by means of a sterile camel's hair brush.

b. Wertheim's method. Human blood-serum (from placenta or pleuritic or other effusion may be used) in a fluid and sterile condition is placed in two or three test-tubes. These are heated to 40° C. and inoculated with the material containing the organism, making dilutions from one to another if necessary. To each tube is then added an equal quantity of nutrient (ordinary or 2%) agar thoroughly liquefied and cooled to 40° C. The two are then thoroughly mixed and quickly poured into Petri dishes and placed in the incubator at 38° C. Colonies appear in 24 hours.

c. Rabbit blood-serum may be used either in a fluid or solid condition.

EXERCISE LXXX. MICROCOCCUS INTRACELLULARIS (Weichselbaum) Mig.

DIPLOCOCCUS OF CEREBRO-SPINAL MENINGITIS.

First described in 1887 by Weichselbaum. It is found in the meningeal exudate of certain cases of epidemic cerebro-spinal meningitis and in nasal secretions in a number of cases.

REFERENCES. Weichselbaum: Fortschritte der Medicine, 1887; Councilman: Rept. Mass. State B. of H. 1898; A. 285; H. 138; M. & R. 172; M. & W. 135; P. 516; S. 310.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon:			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:

2. Relation to free oxygen:

3. Relation to other agents, such as:

desiccation, light, disinfectants, etc.:

4. Pigment production:

5. Gas production in glucose media:

a. Shake culture

b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

(3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.

(4) reaction in open arm: (5) gas formula, H : CO_2 : :

6. Acid or alkali production, litmus milk.

7. Reduction of nitrates; to nitrites, to ammonia

8. Indol production; 24 hours 48 hours days

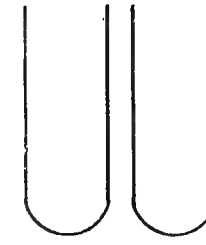
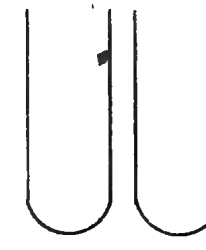
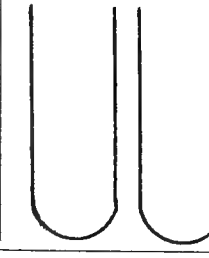
fecal odor; 24 hours 48 hours days

9. Enzyme production: proteolytic diastatic

10. Characteristic odor

11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXXI. SARCINA TETRAGENA (Gaffky) Mig.

MICROCOCCUS TETRAGENUS.

First described in 1883 by Gaffky. It is found in phthisical cavities and sputum and it occasionally occurs in mixed infections as abscesses connected with carious teeth, about the neck, jaws, and middle ear, rarely elsewhere.

REFERENCES. Gaffky: Langenbeck's Archev, 1883, 28: 500. A. 309; H. 139; M. & R. 171; M. & W. 133; McF. 448; P. 472; S. 314.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: .....(2) closed arm: .....

(3) rate of development: 24 hours.....per cent., 48 hours.....per cent., 72 hours .....per cent., ...hours. .....per cent.

(4) reaction in open arm: .....(5) gas formula, H : CO_2 : : : .

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites.....to ammonia.....

8. Indol production; 24 hours.....48 hours.....days.....

fecal odor; 24 hours.....48 hours.....days.....

9. Enzyme production: proteolytic.....diastatic .....

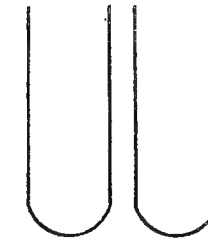
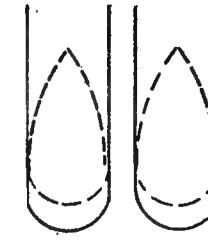
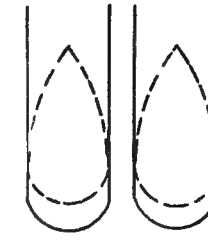
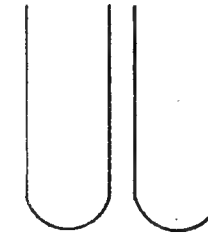
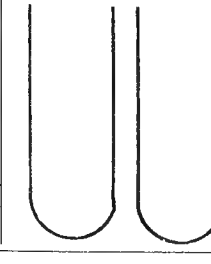
.....

.....

10. Characteristic odor.....

11. Pathogenesis.....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48 ..... Hours.	6 ..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXXII. BACTERIUM ANTHRACIS (Koch) Mig.

BACILLUS OF ANTHRAX.

First described by Robert Koch in 1876. Found in the blood and tissue in cases of anthrax or splenic fever.

REFERENCES. Koch: Cohn's Beitraege zur Biologie der Pflanzen, 1876, 2; 277. Chester: Dept. Delaware Exp. Station, July, 1895. A. 448; H. 151; L. & K. 287; M. & R. 295; M. & W. 156; McF. 356; P. 547; S. 328.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:

2. Relation to free oxygen:

3. Relation to other agents, such as:

desiccation, light, disinfectants, etc:

4. Pigment production:

5. Gas production in glucose media:

a. Shake culture

b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

(3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.

(4) reaction in open arm: (5) gas formula, $H : CO_2$: :

6. Acid or alkali production, litmus milk

7. Reduction of nitrates; to nitrites, to ammonia

8. Indol production; 24 hours, 48 hours days

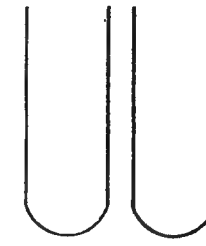
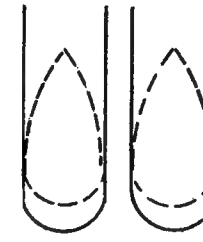
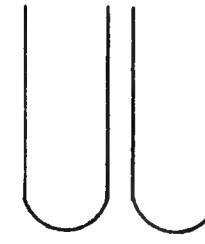
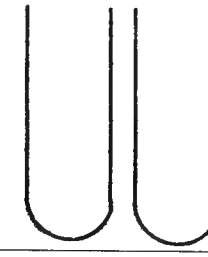
fecal odor; 24 hours, 48 hours days

9. Enzyme production: proteolytic diastatic

10. Characteristic odor

11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXXIII. BACTERIUM PNEUMONIAE (Weichselbaum) Mig.

PNEUMOCOCCUS; DIPLOCOCCUS OF PNEUMONIA; MICROCOCCUS LANCEOLATUS.

First described by Sternberg in 1880. Found in saliva and nasal secretion of healthy persons—about 20 per cent. Usually present in "rusty sputum" of pneumonia.

REFERENCES. Weichselbaum: Am. Jour. Med. Sci., July, 1886: Welch: Johns Hop. Hosp. Bulletin, 1892, 3; 125; A. 303. H. 273; L. & K. 118; M.& R. 204; M. & W. 128; McF. 345; P. 498; S. 298.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.

(4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : : .

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ....., to ammonia .....

8. Indol production; 24 hours ....., 48 hours ..... days .....

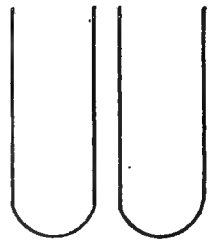
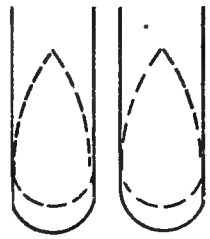
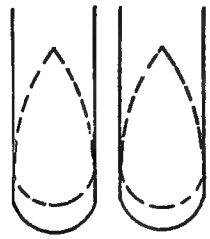
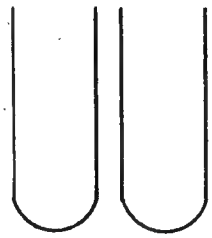
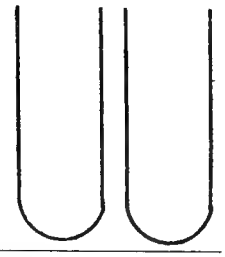
fecal odor; 24 hours ....., 48 hours ..... days .....

9. Enzyme production: proteolytic ..... diastatic .....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXXIV. BACTERIUM PNEUMONICUM (Friedlander) Mig.

FRIEDLANDER'S BACILLUS.

First described by Friedlander in 1882. Found frequently in normal saliva, lungs, "rusty sputum" of pneumonia, and has been found in air and water.

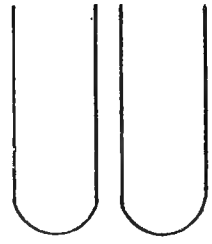
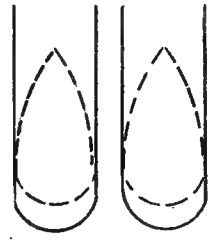
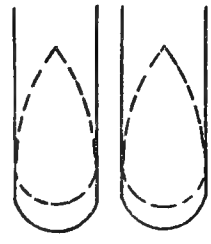
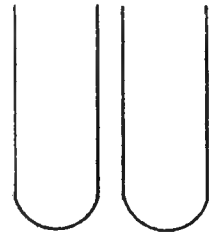
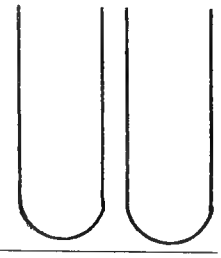
REFERENCES. Friedlander: Virchow's Archiv, 32; 319; H. 278; L. & K. 119; M. & R. 211; McF. 352; P. 458; S. 296.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc.:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.
   (4) reaction in open arm: (5) gas formula, H : CO_2 : :
6. Acid or alkali production, litmus milk.
7. Reduction of nitrates; to nitrites, to ammonia
8. Indol production; 24 hours, 48 hours, days
   fecal odor; 24 hours, 48 hours, days
9. Enzyme production: proteolytic diastatic
10. Characteristic odor
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXXV. BACTERIUM CUNICULICIDA Koch.

BACILLUS OF CHICKEN CHOLERA; BACILLUS OF SWINE PLAGUE; BACILLUS SEPTICAEMIAE HEMORRHAGICAE.

First described by Koch in 1878. Found in blood, organs and excreta of chickens suffering with fowl cholera, and swine suffering from swine plague.

REFERENCES. Koch: Wundinfektionskrankheiten. Septikaemie bei Kaninchen, 1878; Smith: Report on Swine Plague, Bureau of Animal Industry, U. S. Dept. Agri., 1891; Smith & Moore: Bull. 6, B. A. I., 1894; H. 268; McF. 409; S. 408.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:

2. Relation to free oxygen:

3. Relation to other agents, such as:

desiccation, light, disinfectants, etc.:

4. Pigment production:

5. Gas production in glucose media:

a. Shake culture

b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

(3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.

(4) reaction in open arm: (5) gas formula, $H : CO_2$: :

6. Acid or alkali production, litmus milk

7. Reduction of nitrates; to nitrites, to ammonia

8. Indol production; 24 hours, 48 hours days

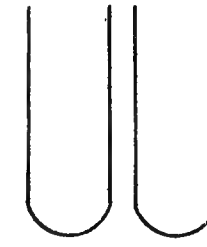
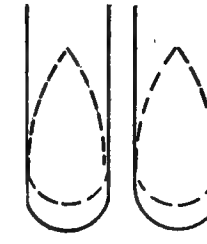
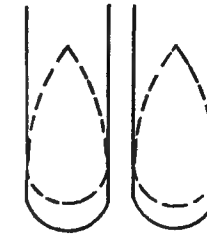
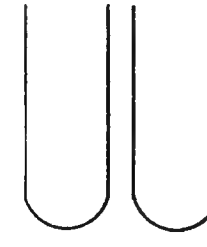
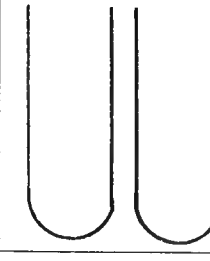
fecal odor; 24 hours, 48 hours days

9. Enzyme production: proteolytic diastatic

10. Characteristic odor

11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXXVI. BACTERIUM RHUSIOPATHIAE (Kitt) Mig.

BACILLUS OF SWINE ERYSIPELAS: ROUGET.

First described by Loeffler in 1882. Found in blood, internal organs, etc., of swine infected with the disease.

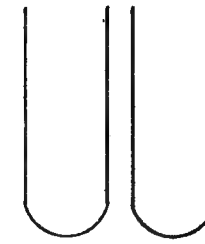
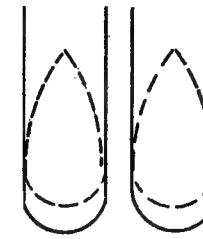
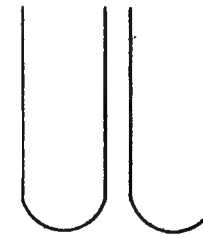
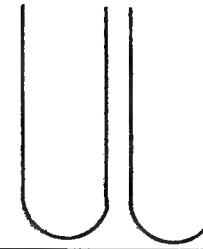
REFERENCES. Loeffler: Arb. aus dem Kaiserl. Gesundheitsamte, 1885, 1; 46; McF. 426; S. 420.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.
   (4) reaction in open arm: (5) gas formula, H : CO_2 : :
6. Acid or alkali production, litmus milk
7. Reduction of nitrates; to nitrites, to ammonia
8. Indol production; 24 hours, 48 hours days
   fecal odor; 24 hours, 48 hours days
9. Enzyme production: proteolytic diastatic
10. Characteristic odor
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24 ..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: a) Surface Colonies. b) Deep Colonies.					
(2) Agar plate: a) Surface Colonies. b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXXVII. BACTERIUM TUBERCULOSIS (Koch) Mig.

BACILLUS OF TUBERCULOSIS.

First described by Koch in 1882. Found in diseased tissue of man and animals and phthisical sputum.

REFERENCES. Koch: Berlin. Klin. Wochenschr., 1882, 15; 221; Smith: Jour. Exp. Med., 1898, 3: 451; A. 312; H. 189; L. & K. 251; M. & R., 224; M. & W. 148; McF. 208; P. 263; S. 375.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:

2. Relation to free oxygen:

3. Relation to other agents, such as:

desiccation, light, disinfectants, etc.:

4. Pigment production:

5. Gas production in glucose media:

a. Shake culture

b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

(3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.

(4) reaction in open arm: (5) gas formula, H : CO_2 : :

6. Acid or alkali production, litmus milk

7. Reduction of nitrates; to nitrites , to ammonia

8. Indol production; 24 hours, 48 hours days

fecal odor; 24 hours, 48 hours days

9. Enzyme production: proteolytic diastatic

10. Characteristic odor

11. Pathogenesis

B. tuberculosis does not grow upon the ordinary artificial media, but may be grown upon blood serum [see p. 89 (1)] and bouillon, agar and potato to which 5% of glycerine has been added. The tubercle bacterium is very sensitive to temperature variations and should therefore be kept at a temperature varying at most only a degree or two from 38° C. It is also extremely sensitive towards desiccation and for this reason the cotton plug should be well paraffined or replaced by a cork through which a small cotton plugged glass tube passes and the incubator kept saturated with moisture. For methods of culture and isolation see Smith: Jour. Exp. Med., 1898, 3; 456.

EXERCISE LXXXVIII. BACTERIUM MALLEI (Loeffler) Mig.

BACILLUS OF GLANDERS.

First described by Loeffler in 1886. Found in the nodules, ulcers, discharges, etc., of glanders or farcy.

REFERENCES. Loeffler: Arbeit. aus dem Kais. Gesundheitsamte, 1886, 1; 141: A. 339; H. 217; L. & K. 300; M. & R. 268; M. & W. 164; McF. 248; P. 598; S. 396.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:

2. Relation to free oxygen:

3. Relation to other agents, such as:

desiccation, light, disinfectants, etc.:

4. Pigment production:

5. Gas production in glucose media:

a. Shake culture

b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

(3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.

(4) reaction in open arm: (5) gas formula, H : CO_2 : :

6. Acid or alkali production, litmus milk.

7. Reduction of nitrates; to nitrites, to ammonia

8. Indol production; 24 hours, 48 hours, days

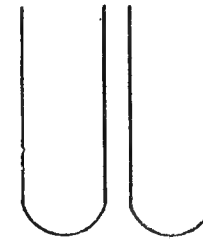
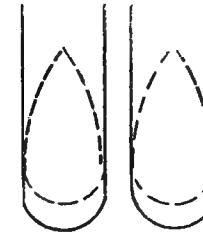
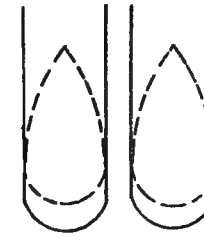
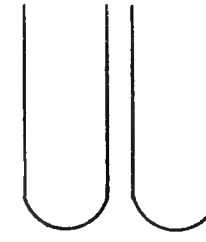
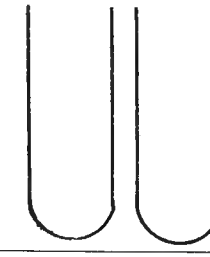
fecal odor; 24 hours, 48 hours, days

9. Enzyme production: proteolytic diastatic

10. Characteristic odor

11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE LXXXIX. BACTERIUM DIPHTHERIAE (Loeffler) Mig.

BACILLUS OF DIPHTHERIA; KLEBS–LOEFFLER BACILLUS.

First described in 1883 by Klebs. First cultivated in 1884 by Loeffler. Found in the false membrane in cases of diphtheria and in small numbers in spleen, liver, etc.; occasionally in healthy throats.

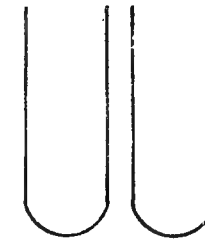
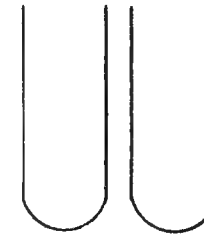
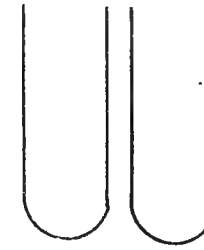
REFERENCES. Klebs: Verhandl. d Kongress fuer innere Medizin, 1883, II. Loeffler: Mitth. aus dem Kais. Gesundheitsamte, 1884, 2; 421. A. 349; H. 162; L. & K. 207; M. & R. 353; M. & W. 137; McF. 284; P. 329; S. 356.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....
.....
2. Relation to free oxygen: .....
3. Relation to other agents, such as: .....
   desiccation, light, disinfectants, etc.: .....
4. Pigment production: .....
.....
5. Gas production in glucose media:
   a. Shake culture .....
   b. Fermentation tube, growth in: (1) open arm ..... (2) closed arm: .....
   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.
   (4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : : .
6. Acid or alkali production, litmus milk .....
7. Reduction of nitrates; to nitrites ..... , to ammonia ....
8. Indol production; 24 hours ..... , 48 hours ..... days .....
   fecal odor; 24 hours ..... , 48 hours ..... days .....
9. Enzyme production: proteolytic ..... diastatic ....
.....
.....
10. Characteristic odor .....
11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE XC. BACTERIUM INFLUENZAE (R. Pfeiffer) Lehm. & Neum.

BACILLUS OF INFLUENZA; LA GRIPPE.

First described in 1892 by R. Pfeiffer. Found in the sputum and nasal secretions of the diseased.

REFERENCES. Pfeiffer: Z. f. H. 1893, 13; 357; A. 334; H. 280; L. & K. 281; M. & R. 431; M. & W. 162; McF. 446; P. 320; S. 370.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:

2. Relation to free oxygen:

3. Relation to other agents, such as:

desiccation, light, disinfectants, etc:

4. Pigment production:

5. Gas production in glucose media:

a. Shake culture

b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

(3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.

(4) reaction in open arm: (5) gas formula, H : CO_2 : :

6. Acid or alkali production, litmus milk

7. Reduction of nitrates; to nitrites, to ammonia

8. Indol production; 24 hours, 48 hours days

fecal odor; 24 hours, 48 hours days

9. Enzyme production: proteolytic diastatic

10. Characteristic odor

11. Pathogenesis

B. influenzae does not grow on the ordinary artificial culture media but may be cultivated on agar slopes upon the surface of which blood has been smeared. The blood from man, rabbits, guinea-pigs and frogs can be used, but that from pigeons is best. The blood may be obtained from a needle prick and spread over the medium with a loop. The skin should first be washed with alcohol and then ether and the first drops should not be used. The sterility of these tubes should be tested by placing them in an incubator for 24 hours previous to inoculation.

EXERCISE XCI. BACILLUS TYPHOSUS Gaffky.

BACILLUS OF TYPHOID FEVER; EBERTH'S BACILLUS.

First described by Eberth in 1880, first cultivated by Gaffky, 1884. It is found in the faeces and urine of typhoid patients.

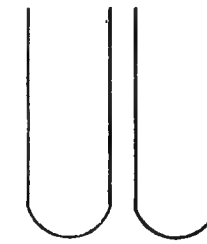
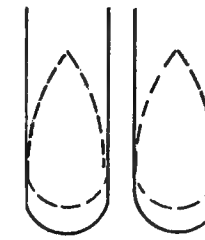
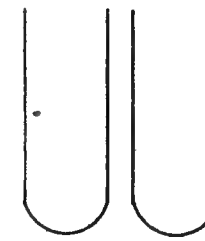
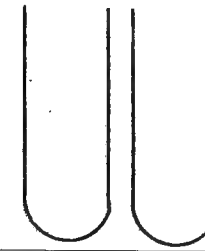
REFERENCES. Eberth: Virchow's Archiv. 1880, 81; 58 and 1881, 83; 486. Gaffky: Mitth. aus dem Kais. Gesundheitsamte, 1884, 2; 372; A. 369; H. 223; L. & K. 166; M. & R. 317; M. & W. 141; McF. 366; P. 402; S. 337.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc.			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....
2. Relation to free oxygen: .....
3. Relation to other agents, such as: .....
   desiccation, light, disinfectants, etc.: .....
4. Pigment production: .....
5. Gas production in glucose media:
   a. Shake culture .....
   b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....
   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.
   (4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : :
6. Acid or alkali production, litmus milk .....
7. Reduction of nitrates; to nitrites ....., to ammonia .....
8. Indol production; 24 hours ....., 48 hours ..... days
   fecal odor; 24 hours ....., 48 hours ..... days
9. Enzyme production: proteolytic ..... diastatic .....
10. Characteristic odor. .....
11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE XCII. BACILLUS PESTIS Kitasato and Yersin.

BACILLUS OF BUBONIC PLAGUE.

Described at about the same time independently by Kitasato and Yersin in 1894. Found in the buboes, and occasionally in the fæces, urine and blood and, in the pneumonic form, in the blood.

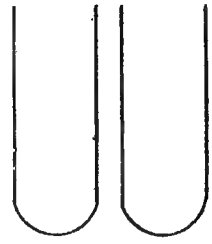
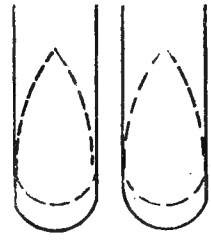
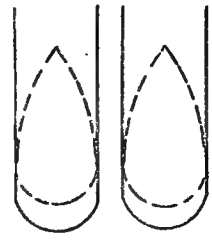
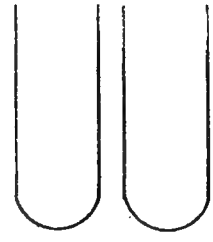
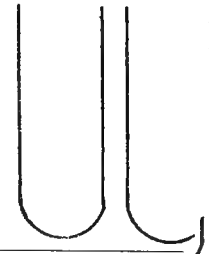
REFERENCES. Kitasato: Lancet, 1894, 2; 428; Yersin: Ann. de l' Inst. Past., 1894, 8; 662; A. 292; H. 259; L. & K. 200; M. & R. 437; McF. 433; P. 606.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature :
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc.:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.
   (4) reaction in open arm: (5) gas formula, H : CO_2 : : :
6. Acid or alkali production, litmus milk.
7. Reduction of nitrates; to nitrites, to ammonia
8. Indol production; 24 hours , 48 hours , days
   fecal odor; 24 hours , 48 hours , days
9. Enzyme production : proteolytic diastatic
10. Characteristic odor
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE XCIII. BACILLUS SUIPESTIFER Kruse.

BACILLUS OF HOG CHOLERA.

First described by Klein, 1884, first cultivated by Salmon and Smith in 1885. Occurs in blood, organs and intestinal contents of hogs suffering from hog cholera.

REFERENCES. Salmon & Smith: Rept Bureau Anim. Ind., 1885-91; H. 269; McF. 413; S. 413.

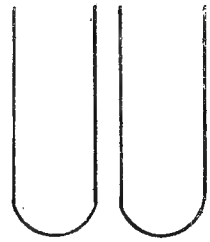
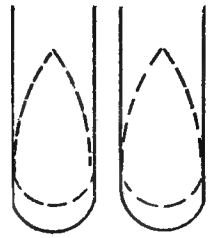
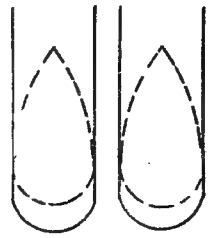
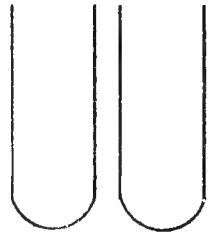
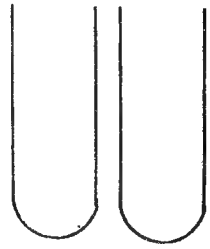
MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc.:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: ...(2) closed arm:
   (3) rate of development: 24 hours ...per cent., 48 hours ...per cent., 72 hours...per cent., ...hours...per cent.
   (4) reaction in open arm: ...(5) gas formula, $H : CO_2$: :
6. Acid or alkali production, litmus milk
7. Reduction of nitrates; to nitrites..., to ammonia
8. Indol production; 24 hours..., 48 hours... days
   fecal odor; 24 hours..., 48 hours... days
9. Enzyme production: proteolytic...diastatic
   Characteristic odor
   Pathogenesis

11.

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C.)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE XCIV. BACILLUS ICTEROIDES Sanarelli.

First described in 1897 by Sanarelli, and claimed by him to be the cause of yellow fever.

REFERENCES. H. 369; M. & R. 453; McF. 400; P. 609.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

   desiccation, light, disinfectants, etc: .....

4. Pigment production: .....

5. Gas production in glucose media:

   a. Shake culture .....

   b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.

   (4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : :

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ....., to ammonia .....

8. Indol production; 24 hours ....., 48 hours ..... days .....

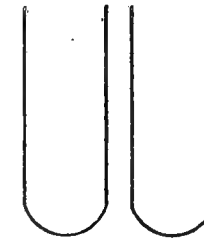
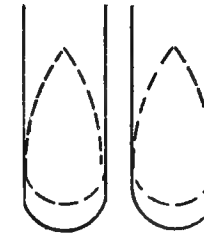
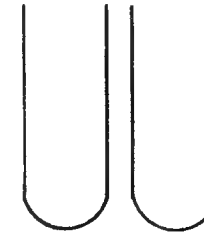
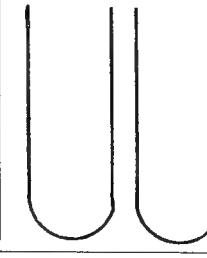
   fecal odor; 24 hours ....., 48 hours ..... days .....

9. Enzyme production: proteolytic ..... diastatic .....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE XCV. PSEUDOMONAS AERUGINOSA (Schroeter) Mig.

BACILLUS PYOCYANEUS OR BACILLUS OF BLUE-GREEN PUS.

First described in 1872 by Schroeter. Found in green pus, and is widely distributed in nature.

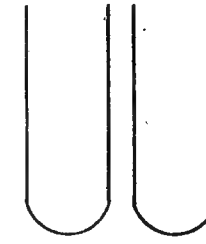
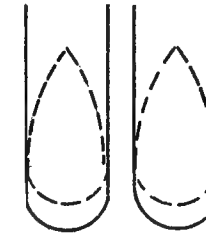
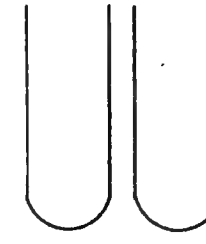
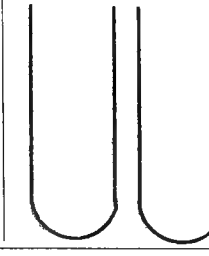
REFERENCES. Schroeter: Cohn's Beitraege zur Biologie, 1872, 1; 126. Barker: Jour. Am. Med. Asso., 1897, July 31. Jordan: Jour. Exp. Med. 1899, 627. Lartigan, *Ibid.*, 1898; 595; A. 287; H. 138; L. & K. 120; M. & R. 170; M. & W. 160; McF. 197; P. 535; S. 454.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....
2. Relation to free oxygen: .....
3. Relation to other agents, such as: .....
   desiccation, light, disinfectants, etc.: .....
4. Pigment production: .....
5. Gas production in glucose media:
   a. Shake culture .....
   b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....
   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.
   (4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : :
6. Acid or alkali production, litmus milk .....
7. Reduction of nitrates; to nitrites ....., to ammonia .....
8. Indol production; 24 hours ....., 48 hours ..... days .....
   fecal odor; 24 hours ....., 48 hours ..... days .....
9. Enzyme production: proteolytic ..... diastatic .....
10. Characteristic odor .....
11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE XCVI. MICROSPIRA COMMA (Koch) Schroeter.

COMMA BACILLUS; CHOLERA VIBRIO.

First described by Koch in 1884. Found in the intestinal contents of cholera patients and has also been isolated several times from a water supply.

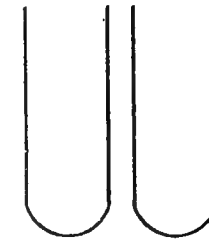
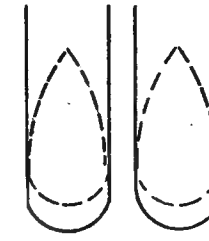
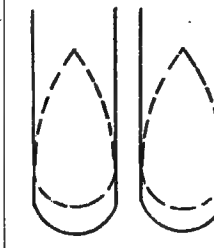
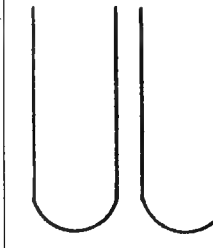
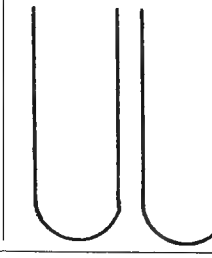
REFERENCES. Koch: Berl. Klin. Wochenschr. 1884, no. 31 u. 32: A. 401; H. 244; L. & K. 181; M. & R. 402; M. & W. 152; McF. 311; P. 568; S 500.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....
2. Relation to free oxygen: .....
3. Relation to other agents, such as: .....
   desiccation, light, disinfectants, etc.: .....
4. Pigment production: .....
5. Gas production in glucose media:
   a. Shake culture .....
   b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....
   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.
   (4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : : .
6. Acid or alkali production, litmus milk. .....
7. Reduction of nitrates; to nitrites ....., to ammonia .....
8. Indol production, 24 hours ....., 48 hours ....., ... days .....
   fecal odor; 24 hours ....., 48 hours ....., ... days .....
9. Enzyme production: proteolytic ..... diastatic .....
10. Characteristic odor .....
11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE XCVII. MICROSPIRA METSCHNIKOVI Mig.

VIBRIO METSCHNIKOVI.

First described in 1888 by Gamaleia. Found in intestinal contents, blood and organs of chickens suffering from a disease resembling chicken cholera.

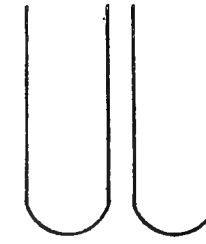
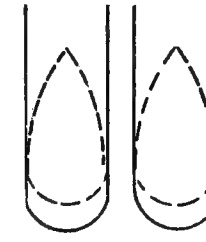
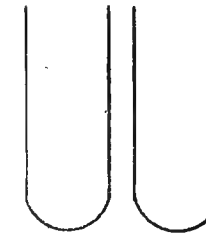
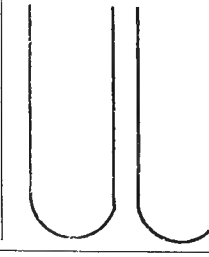
REFERENCES. Gamaleia: Ann. d l' Inst, Past. 1888, 2; 482. A. 441; H. 256; M. & R. 426; McF. 332; P. 593; S. 511.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc.:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.
   (4) reaction in open arm: (5) gas formula, $H : CO_2$: : :
6. Acid or alkali production, litmus milk.
7. Reduction of nitrates; to nitrites, to ammonia.
8. Indol production; 24 hours, 48 hours ..... days
   fecal odor; 24 hours, 48 hours ..... days
9. Enzyme production: proteolytic diastatic
10. Characteristic odor.
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE XCVIII. MICROSPIRA FINKLERI Schroeter.

SPRILLUM OF FINKLER AND PRIOR.

First described in 1884 by Finkler & Prior. Deutsche Med. Wochenschr., 1884, 632.

REFERENCES. A. 429; H. 257; M. & R. 428; McF. 326; P. 589; S 509.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:

2. Relation to free oxygen:

3. Relation to other agents, such as:

desiccation, light, disinfectants, etc:

4. Pigment production:

5. Gas production in glucose media:

a. Shake culture

b. Fermentation tube, growth in: (1) open arm: (2) closed arm:

(3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.

(4) reaction in open arm: (5) gas formula, H : CO_2 : :

6. Acid or alkali production, litmus milk

7. Reduction of nitrates; to nitrites, to ammonia

8. Indol production; 24 hours, 48 hours days

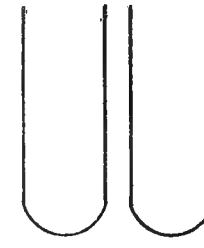
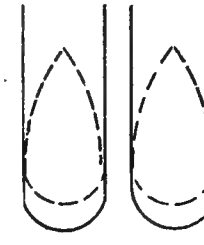
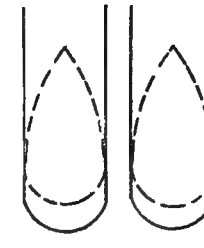
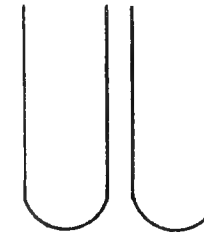
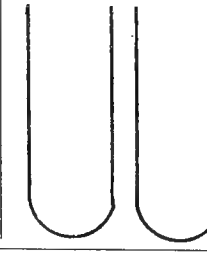
fecal odor; 24 hours, 48 hours days

9. Enzyme production: proteolytic diastatic

10. Characteristic odor

11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Name of organism .....

Source, habitat, etc. .....

.....

References .....

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon	.....	.....	
.....	.....	.....	
b. Agar	.....	.....	
.....	.....	.....	
c. Gelatin	.....	.....	
.....	.....	.....	
d. Other media	.....	.....	
2. Size	.....	.....	
3. Cell groupings	.....	.....	
and arrangements	.....	.....	
in growths	.....	.....	
4. Staining powers:	.....	.....	
a. Aqueous gentian-violet	.....	.....	
b. Loeffler's methylen-blue	.....	.....	
c. Gram's stain	.....	.....	
d. Special stains	.....	.....	
5. Motility:	.....	.....	
a. Character of movement	.....	.....	
b. Flagella stain	.....	.....	
6. Spores	.....	.....	
.....	.....	.....	
7. Special characters, such as:	.....	.....	
deposits, vacuoles	.....	.....	
pleomorphic and involution forms, capsules, etc	.....	.....	

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.

(4) reaction in open arm: ..... (5) gas formula, $H : CO_2$: : : .

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ....., to ammonia .....

8. Indol production; 24 hours ....., 48 hours ..... days .....

fecal odor; 24 hours ....., 48 hours ..... days. .....

9. Enzyme production: proteolytic ..... diastatic .....

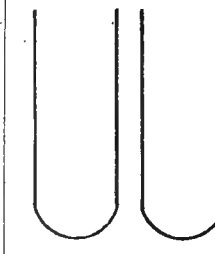
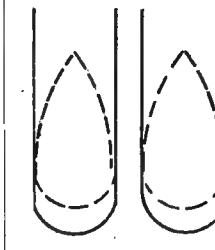
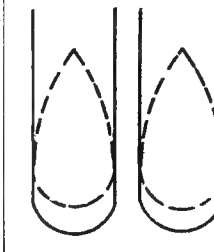
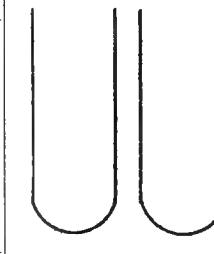
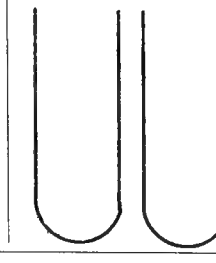
.....

.....

10. Characteristic odor. .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24 ..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Name of organism.....

Source, habitat, etc.....

.....

References .....

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: .....(2) closed arm: .....

(3) rate of development: 24 hours .. per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours..... per cent.

(4) reaction in open arm: .....(5) gas formula, H : CO_2 : : :

6. Acid or alkali production, litmus milk. .....

7. Reduction of nitrates; to nitrites..... to ammonia.....

8. Indol production; 24 hours ..... 48 hours..... days.....

fecal odor; 24 hours ..... 48 hours..... days.....

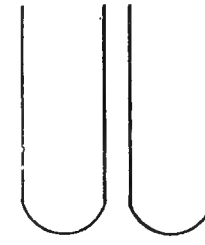
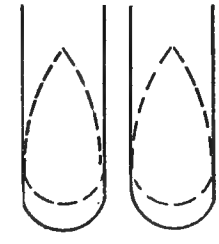
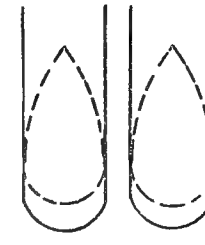
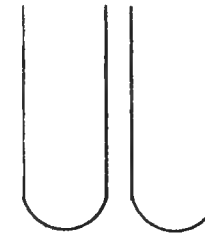
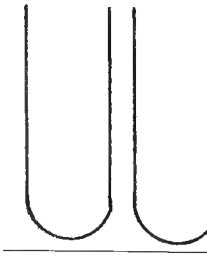
9. Enzyme production : proteolytic ..... diastatic.....

.....

10. Characteristic odor. .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Name of organism .....

Source, habitat, etc. .....

.....

References .....

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon			
.....			
b. Agar			
.....			
c. Gelatin			
.....			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
.....			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.

(4) reaction in open arm: ..... (5) gas formula, $H : CO_2$: : : .

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ..... to ammonia .....

8. Indol production; 24 hours ..... 48 hours ..... days .....

fecal odor; 24 hours ..... 48 hours ..... days .....

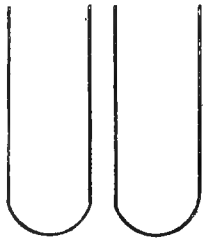
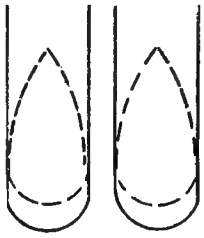
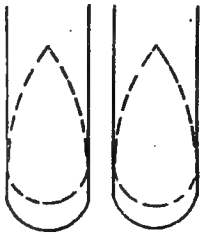
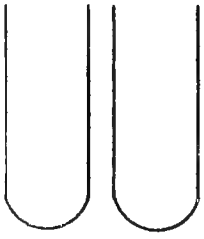
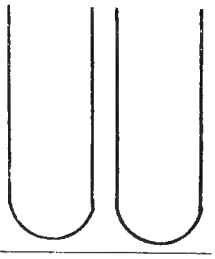
9. Enzyme production: proteolytic ..... diastatic .....

.....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Same of organism .....

Source, habitat, etc. .....

.....

References.....

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon			
.....			
b. Agar			
.....			
c. Gelatin			
.....			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
.....			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.

(4) reaction in open arm: ..... (5) gas formula, $H : CO_2$: : : .

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ..... , to ammonia .....

8. Indol production; 24 hours ..... , 48 hours ..... days .....

fecal odor; 24 hours ..... , 48 hours ..... days .....

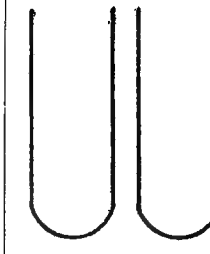
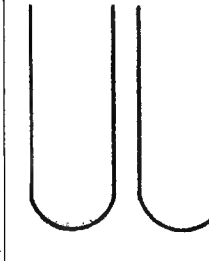
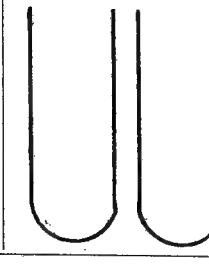
9. Enzyme production: proteolytic ..... diastatic .....

.....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Name of organism .....

Source, habitat, etc. .....

.....

References .....

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon	.....	.....	
.....	.....	.....	
b. Agar	.....	.....	
.....	.....	.....	
c. Gelatin	.....	.....	
.....	.....	.....	
d. Other media	.....	.....	
2. Size	.....	.....	
3. Cell groupings	.....	.....	
and arrangements	.....	.....	
in growths	.....	.....	
4. Staining powers:	.....	.....	
a. Aqueous gentian-violet	.....	.....	
b. Loeffler's methylen-blue	.....	.....	
c. Gram's stain	.....	.....	
d. Special stains	.....	.....	
5. Motility:	.....	.....	
a. Character of movement	.....	.....	
b. Flagella stain	.....	.....	
6. Spores	.....	.....	
.....	.....	.....	
7. Special characters, such as:	.....	.....	
deposits, vacuoles	.....	.....	
pleomorphic and involution forms, capsules, etc	.....	.....	

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours .... per cent.

(4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : : .

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ....., to ammonia .....

8. Indol production; 24 hours ....., 48 hours ..... days .....

fecal odor; 24 hours ....., 48 hours ..... days .....

9. Enzyme production: proteolytic ..... diastatic .....

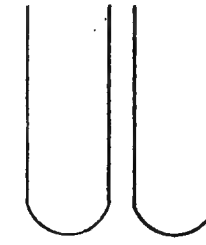
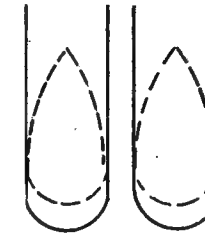
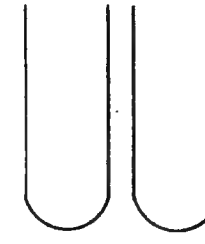
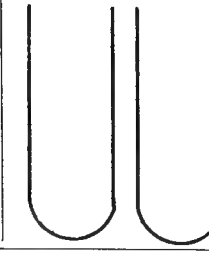
.....

.....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

CHAPTER VII.

PATHOGENIC ANAEROBES.

Anaerobic bacteria may be furnished conditions, which permit of their development, in a variety of ways and a very considerable number of pieces of apparatus have been devised to secure this end. In a general way all of the methods may be grouped under the following heads:

1. Displacement of air.
2. Absorption of oxygen.
3. Exhaustion of air.
4. Exclusion of air.
5. Miscellaneous methods, in the presence of reducing substances as litmus, or a strongly aerobic germ, etc.

The first two methods are the most reliable. In the displacement method, hydrogen, carbon dioxide or illuminating gas may be used; hydrogen is best. This gas is readily prepared by the action of sulphuric acid (1:8) on zinc. Either a Kipp generator may be used or one of a simpler construction. The gas should be washed, 1st. in lead nitrate to absorb the sulphuretted hydrogen, 2nd. in silver sulphate to absorb any arseniuretted or phosphuretted hydrogen, and 3rd. in potassium hydrate to remove sulphur and carbon dioxide.

The cultures are made in media containing glucose (which should preferably be freshly prepared and always boiled immediately before being inoculated), either as test-tube or plate cultures. Novy's anaerobic jars are perhaps the most satisfactory receptacles for the cultures. (For careful description of same, see N. 306.)

In the second method (Buchner's method) an alkaline solution of pyrogallic acid is used to absorb the oxygen. The cultures may be placed in Novy jars or similar receptacles; for tube cultures a large wide mouthed bottle fitted with a rubber cork does very well. The dry pyrogallic acid is placed in the bottom of the receptacles, about 1 gram to every 100 cc. of air space, the tubes are put in place, then about 10 cc. of a normal sodium hydroxide is added to each gram of pyrogallic acid, and the apparatus immediately and hemetically sealed.

References. A. 206; L. & K. 98; M. & R. 68; M. & W. 117; McF. 153; P. 233; S. 78.

EXERCISE XCIX. BACTERIUM WELCHII Mig.

BACILLUS AEROGENES CAPSULATUS.

First described by Welch in 1892. Occurs at autopsies in which gas bubbles are present in the larger vessels, accompanied by the formation of numerous small cavities in the liver containing gas. It has been found also in emphysematous phlegmons, in puerperal sepsis, in peritonitis and in other conditions (M. & W.). Widely distributed in nature. (Welch.)

REFERENCES. Welch and Nuttall: Bull. Johns Hopkins Hospital, 1892, 3; 81; Welch & Flexner: Jour. Exp. Med., 1896, 1; 5; H. 140; M. & W. 173; McF. 463; P. 545; S. 731.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent.

(4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : :

6. Acid or alkali production, litmus milk. .....

7. Reduction of nitrates; to nitrites ....., to ammonia. .....

8. Indol production; 24 hours ....., 48 hours ..... days .....

fecal odor; 24 hours ....., 48 hours ..... days .....

9. Enzyme production: proteolytic ..... diastatic .....

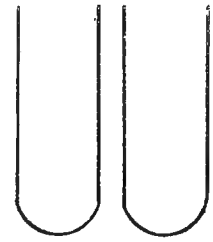
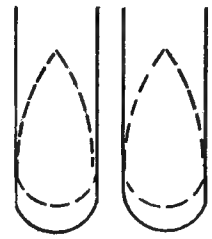
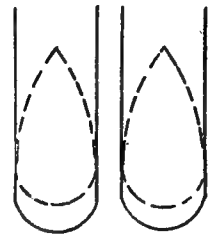
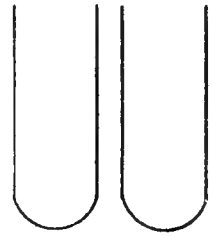
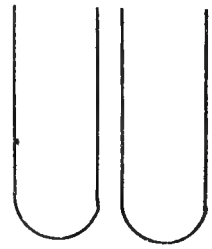
.....

.....

10. Characteristic odor. .....

11. Pathogenesis. .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE C. BACILLUS CHAUVAEI Arloing, Cornevin and Thomas.

BACILLUS OF SYMPTOMATIC ANTHRAX.

First described by Arloing, Cornevin and Thomas in 1887. It occurs in the subcutaneous tissue, muscles and serous exudate of animals suffering from symptomatic anthrax.

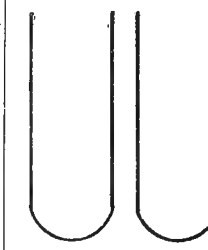
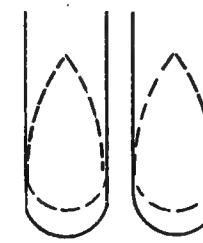
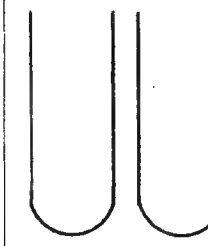
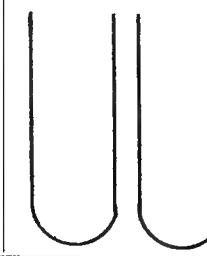
REFERENCES. Arloing, Cornevin and Thomas; Le Charbon symptomatique du baeuf, 2nd edit. Paris, 1887; A. 482; H. 304; McF. 453; P. 563; S. 493.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature:
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cent.
   (4) reaction in open arm: (5) gas formula, H : CO_2 : :
6. Acid or alkali production, litmus milk
7. Reduction of nitrates; to nitrites, to ammonia
8. Indol production; 24 hours, 48 hours days
   fecal odor; 24 hours, 48 hours days
9. Enzyme production: proteolytic diastatic
10. Characteristic odor
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE CI. BACILLUS OEDEMATIS Liborius.

BACILLUS OF MALIGNANT OEDEMA.

First described by Pasteur in 1877. Widely distributed in soil and putrefying material. Few cases on record of infection of ma

REFERENCES. Z. f. H., 1886; 1:158; A. 476; H. 302; L. & K. 305; M. & R. 394; M. & W. 175; M. & W. 459; P. 543; S.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature: .....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cen

(4) reaction in open arm: ..... (5) gas formula, H : CO_2 : : :

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ....., to ammonia .....

8. Indol production; 24 hours ....., 48 hours ..... days .....

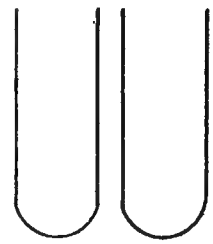
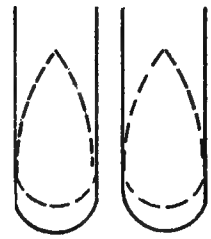
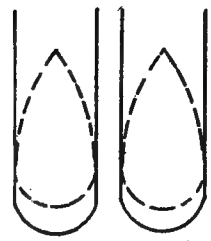
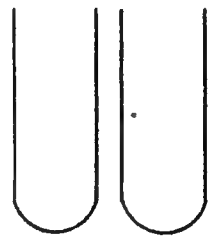
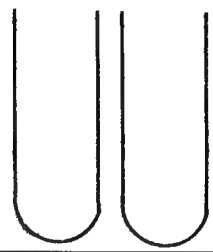
fecal odor; 24 hours ....., 48 hours ..... days .....

9. Enzyme production: proteolytic ..... diastatic .....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

EXERCISE CII. BACILLUS TETANI Nicolaier.

Discovered by Nicolaier, 1884. First cultivated by Kitasato, 1889. Occurs in man and animals suffering from the disease and wide distributed in nature, especially in soil.

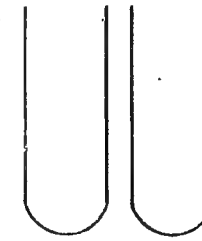
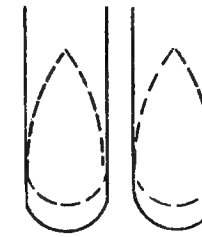
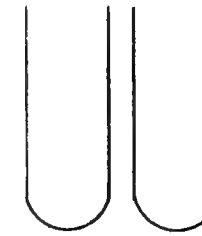
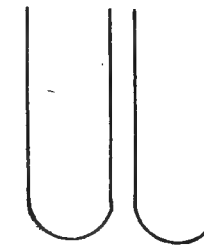
REFERENCES. Nicolaier: Deutszche Med. Wochenschrift, 1884; Kitasato: Deutsche Med. Wochenschrift, 1889; A. 469; H. 2 L. & K. 230; M. & R. 376; M. & W. 171; McF. 274; P. 385; S. 482.

MORPHOLOGICAL CHARACTERS.	Age of Cultures.	Incubation temp. (°C.)	SKETCHES.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

PHYSIOLOGICAL CHARACTERS.

1. Relation to temperature :
2. Relation to free oxygen:
3. Relation to other agents, such as:
   desiccation, light, disinfectants, etc.:
4. Pigment production:
5. Gas production in glucose media:
   a. Shake culture
   b. Fermentation tube, growth in: (1) open arm: (2) closed arm:
   (3) rate of development: 24 hours per cent., 48 hours per cent., 72 hours per cent., hours per cen
   (4) reaction in open arm: (5) gas formula, H : CO_2 : :
6. Acid or alkali production, litmus milk.
7. Reduction of nitrates; to nitrites, to ammonia
8. Indol production; 24 hours , 48 hours, , days
   fecal odor; 24 hours , 48 hours, , days
9. Enzyme production : proteolytic diastatic
10. Characteristic odor
11. Pathogenesis

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Name of organism .....

Source, habitat, etc. .....

.....

References.... .....

Morphological Characters.	Age of Cultures.	Incubation temp. (oC.)	Sketches.
1. Form:			
a. Bouillon	.....	.....	
.....	.....	.....	
b. Agar	.....	.....	
.....	.....	.....	
c. Gelatin	.....	.....	
.....	.....	.....	
d. Other media	.....	.....	
2. Size	.....	.....	
3. Cell groupings	.....	.....	
and arrangements	.....	.....	
in growths	.....	.....	
4. Staining powers:	.....	.....	
a. Aqueous gentian-violet	.....	.....	
b. Loeffler's methylen-blue	.....	.....	
c. Gram's stain	.....	.....	
d. Special stains	.....	.....	
5. Motility:	.....	.....	
a. Character of movement	.....	.....	
b. Flagella stain	.....	.....	
6. Spores	.....	.....	
.....	.....	.....	
7. Special characters, such as:	.....	.....	
deposits, vacuoles	.....	.....	
pleomorphic and involution forms, capsules, etc	.....	.....	

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

desiccation, light, disinfectants, etc.: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

a. Shake culture .....

b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

(3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent

(4) reaction in open arm: ..... (5) gas formula, $H : CO_2$: : :

6. Acid or alkali production, litmus milk. .....

7. Reduction of nitrates; to nitrites ..... , to ammonia .....

8. Indol production; 24 hours ..... , 48 hours ..... days .....

fecal odor; 24 hours ..... , 48 hours ..... days .....

9. Enzyme production: proteolytic ..... diastatic .....

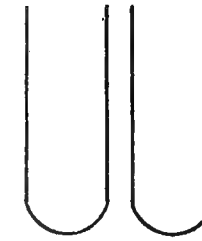
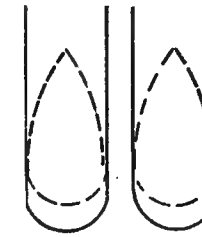
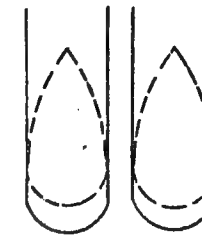
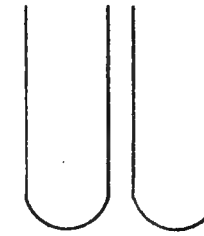
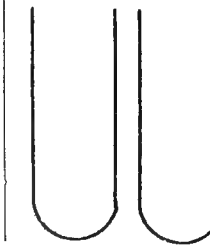
.....

.....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
(1) Gelatin plate: (a) Surface Colonies. (b) Deep Colonies.					
(2) Agar plate: (a) Surface Colonies. (b) Deep Colonies.					
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

Name of organism .....

Source, habitat, etc. .....

.....

References .....

Morphological Characters.	Age of Cultures.	Incubation temp. (°C.)	Sketches.
1. Form:			
a. Bouillon			
b. Agar			
c. Gelatin			
d. Other media			
2. Size			
3. Cell groupings			
and arrangements			
in growths			
4. Staining powers:			
a. Aqueous gentian-violet			
b. Loeffler's methylen-blue			
c. Gram's stain			
d. Special stains			
5. Motility:			
a. Character of movement			
b. Flagella stain			
6. Spores			
7. Special characters, such as:			
deposits, vacuoles			
pleomorphic and involution forms, capsules, etc			

Physiological Characters.

1. Relation to temperature: .....

.....

2. Relation to free oxygen: .....

3. Relation to other agents, such as: .....

   desiccation, light, disinfectants, etc: .....

4. Pigment production: .....

.....

5. Gas production in glucose media:

   a. Shake culture .....

   b. Fermentation tube, growth in: (1) open arm: ..... (2) closed arm: .....

   (3) rate of development: 24 hours ..... per cent., 48 hours ..... per cent., 72 hours ..... per cent., ... hours ..... per cent

   (4) reaction in open arm: ..... (5) gas formula, $H : CO_2$: : : .

6. Acid or alkali production, litmus milk .....

7. Reduction of nitrates; to nitrites ....., to ammonia .....

8. Indol production; 24 hours ....., 48 hours ..... days .....

   fecal odor; 24 hours ....., 48 hours ..... days .....

9. Enzyme production: proteolytic ..... diastatic .....

.....

.....

10. Characteristic odor .....

11. Pathogenesis .....

Culture Characters.

	Reaction of Medium, Incubation Temp. (°C)	24..... Hours.	48..... Hours.	6..... Days.	Sketches.
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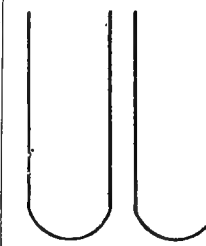
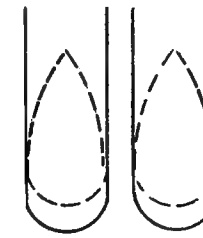
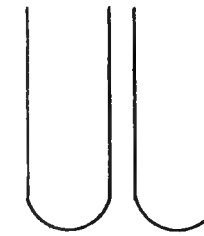
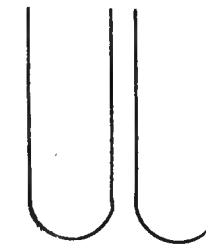
(3) Gelatin Stab.					
(4) Agar Streak.					
(5) Potato.					
(6) Bouillon.					
(7) Special Media.					

CHAPTER VIII.

ANIMAL INOCULATION AND STAINING OF BACTERIA IN TISSUE.

EXERCISE CIII. ANIMAL INOCULATION.

METHODS OF INOCULATION. Animal inoculation is practiced to determine the pathogenic properties of an organism and also the character of the tissue changes produced. The animals commonly used are white mice and rats, rabbits, guinea pigs and pigeons. Inoculations are usually made intraperitoneally, intravenously or subcutaneously, and in special cases into the pleural cavity, brain, eye, etc., etc. Mice require a holder, the inoculation being made at the root of the tail. Other animals can usually be held by an assistant.

Subcutaneous. The place selected is usually the abdominal wall. Pigeons are inoculated in the pectoral muscles; the hair or feathers should be removed and the skin washed with a disinfectant, e. g., 5% carbolic acid.

a. For liquids a sterilized hypodermic syringe is used. A fold of the skin is raised, the needle of the syringe inserted and the requisite amount of culture injected.

b. For solid material a pocket is made which is stitched, or sealed with contractile collodion, after the material is introduced.

Intraperitoneal. Prepare seat of inoculation as above, then plunge needle directly into the peritoneal cavity.

Intravenous. A rabbit is generally chosen for this purpose and the inoculation made into the dorsal vein of the ear. Slight pressure at the base of the ear will render the vein more prominent. Avoid the introduction of air, which causes immediate death, and keep the animals under close observation for one hour.

Inoculation into Lymphatic system. Fluid cultures or suspensions of bacteria can be injected into the lymphatics by way of the testicles, by plunging the point of the needle into the substance of the testicle and injecting the desired amount of fluid.

Inoculation into the Pleural Cavity. Where necessary the needle is introduced into the pleural cavity between the ribs. It is very difficult to perform this experiment without injuring the lung.

Inoculation into the Anterior Chamber of the eye. Rarely practiced. The eye is treated with a few drops of cocaine (2 % solution) and then the needle is inserted through the cornea just in front of its junction with the sclerotic, the needle passing into the anterior chamber in a plane parallel to the plane of the iris.

The following inoculations are those most frequently made:

Streptococcus pyogenes. Mice or rabbits, *intravenous.*

Sarcina tetragena. Guinea pigs and white mice, *subcutaneous.*

Bacterium anthracis. Guinea pigs or rabbits, *subcutaneous.*

—— *pneumoniae.* Rabbits and mice, *subcutaneous.*

—— *pneumonicum.* Mice and young rats, *intraperitoneal.*

—— *tuberculosis.* Guinea pigs, rabbits and field mice, *any method of inoculation* will produce the disease.

B. mallei. Male guinea pigs, *infection of lymphatics.*

—— *diphtheriae.* Guinea pigs, rabbits and fowl, *subcutaneous* and *intratracheal.*

Bacillus pestis. Rats, mice, guinea pigs and rabbits, *subcutaneous.*

—— *suipestifer.* Rabbits and mice, *subcutaneous.*

STERILIZATION OF INSTRUMENTS. These are best sterilized by boiling in a solution of soda or borax for 15 minutes. This is accomplished in an especially designed apparatus or in an ordinary enamel stew pan. In case of emergencies the instruments may be dipped in benzene or alcohol and burned. This is less injurious to the instrument than heating in the direct flame.

Use blank, p. 168, for preservation of data.

OBSERVATION OF INOCULATED ANIMALS. After inoculation the animals should be placed in separate cages, or if placed together they must be described or marked so as to be easily identified. They must also be kept under constant observation and the following conditions noted:

1. Temperature.
2. Loss of Weight.
3. Peculiar position in cage.
4. Loss of appetite.
5. Condition of the coat or hair.
6. Condition of the secretion of the air passages, conjunctiva and kidneys; diarrhea or hemorrhage from the bowels.
7. The condition of the seat of inoculation.

The animals should be fed regularly, weighed at the same hour each day and the temperature taken at the rectum.

POST MORTEM EXAMINATION.

Perform the autopsy as soon as possible after death. When delay cannot be avoided, place the animal in the ice-chest until such time as is convenient.

A.

1. Inspect externally and note presence and character of any lesion.
2. Sterilize a suitable post-mortem board with corrosive sublimate solution, 1 to 1000, place the animal belly upwards and tack the four legs fast to the board.
3. Wash the surface of the thorax and abdomen with corrosive sublimate solution, make an incision through the skin at the pubis, introducing one blade of the scissors, and extend the incision as far as the chin.
4. Carefully dissect the skin away from the abdomen, thorax, axillary, inguinal, and cervical regions, and fore and hind legs, and pin it to the board as far as possible from the thorax and abdomen. It is from the skin that the chances of contamination are greatest.

B.

All incisions from now on are made with sterilized instruments.

1. Take an ordinary potato-knife, heat it quite hot, and place it on the abdomen in the region of the linea alba until the fascia begins to burn; the knife is then held transversely to this line over the center of the abdomen, making two sterilized tracks through which the abdomen may be opened by crucial incisions: two burned lines are also made along the sides of the thorax.

2. Make a central longitudinal incision from the sternum to the genitalia with sterile scissors, the abdominal wall being held up with sterilized forceps, or a hook to prevent the viscera being injured. A transverse incision is made in a similar manner. Cut through the ribs with strong sterilized scissors along the sterilized tracks on the sides of the thorax, when the whole anterior wall of the thorax is easily lifted and entirely removed by severing the diaphragm connections.

3. When the thoracic and abdominal cavities are fully exposed, a careful examination of the organs and surroundings is made without disturbing them.

Culture plates (Petri-dish) or roll cultures are prepared from the blood, liver, spleen, kidneys, and any exudates present.

The method is as follows:

(1) Heat a scalpel and scorch a small surface of the organ from which the cultures are to be made.

(2) Heat the scalpel again and penetrate the capsule of the organ with the point, and through the opening insert a stout sterilized platinum loop, push it into the tissues, twist around, and obtain enough material from the center of the organ to make the culture.

Cultures from blood are usually made from one of the heart cavities, the surface being seared with a hot knife before opening. As soon as the culture material is obtained, cover-glass specimens are prepared from each organ and existing exudates.

Small pieces of each organ are also preserved for future examination.

When the autopsy is finished the remainder of the animal should be burned and the instruments should be sterilized. Wash the post-mortem board with sublimate solution. The cover-glasses and other material likely to contain infectious matter must also be sterilized when of no further use.

Cultures are to be incubated at 38° C., growth examined microscopically, and by means of sub-cultures.

References. The above is taken largely from Bowhill, 74; see also A. 219; N. 260; and other texts.

BLANK FOR ANIMAL EXPERIMENTS.

Animal No. .....

Experimenter .....

Animal .....Sex.....Age.....Weight.....

Specimens received .....o'clock.....M.

Organs .....

Museum No. .....Slide No. .....

Experiment:

Died or killed .....o'clock.....M.

Autopsy .....o'clock.....M.

Findings:

Bacteriological Examination:

Histological Examination:

EXERCISE CIV. PREPARATION OF TISSUE FOR EXAMINATION.

Portions of the diseased tissue, removed at autopsy, should be cut into cubes having edges about 5 mm. long and treated as follows:

1). FIXING. Use 15 or 20 times their volume of 95% alcohol for 24 hrs. The specimens should be placed on cotton to keep them near the top and the alcohol changed after 3 or 4 hours, if they are not to be sectioned immediately carry to 80% alcohol.

Where larger sections are desired they should be left a longer time in the alcohol.

2). PREPARATION FOR SECTIONING.

A.

Paraffin Method.

a. Absolute Alcohol 6-24 hours.

b. Xylene 6-24 hours.

c. Paraffin melting at 50°C. and kept in an oven or water-bath at a temperature a few degrees above the melting point of the paraffin.

d. Embed. Pour melted paraffin into a paper box or other suitable receptacle and with warm forceps, arrange block of tissue in proper position and cool rapidly by plunging into cold water.

B.

Celloidin Method.

a. Mixture of ether and absolute alcohol (equal parts) 24 hours.

b. Thin celloidin (about 6%) 24 hours to several weeks.

c. Thick celloidin (about 12%) 24 hours to several weeks.

d. Remove block of tissue to a piece of wood fiber covered with "thick" celloidin, orient, dry a few minutes in air then place in 80% alcohol for 6-24 hours.

C.

Freezing Method.

a. Place in 1% Formalin 2 hours.

b. Place tissue on plate of freezing microtome in water or better first soak tissue in a syrupy solution of gum-arabic and moisten plate with same before freezing.

3). SECTIONING. Cut sections from 10–12 μ thick.

4). MANIPULATION OF SECTIONS.

a. Celloidin sections can be preserved in 80 % alcohol and are best stained by placing the sections first in water and then in the stain. The various reagents are best used in watch glasses and the sections transferred from one to the other by means of a section lifter.

b. Paraffin sections should be fixed to the slide or cover-glass as follows: A water-bath is heated up to a few degrees below the melting point of the paraffin, the sections are placed on the water where they will straighten out and are then transferred to the slide or more conveniently to the cover-glass by simply dipping the same into the water and drawing up the section by means of the fine point of a pair of forceps or a needle, draining off the water and drying the section in an incubator for a few hours. The sections are more secure if the cover-glasses are first smeared with a thin coat of egg albumin. When the sections are once fixed to the cover the staining can be carried on in the forceps as with ordinary cover-glass preparations. Before staining, however, the paraffin must be removed; this is done with xylene and this in turn removed with *absolute* alcohol.

REFERENCES. A. 173; M. & W. 204–239; N. 531.

EXERCISE CV. STAINING SECTIONS.

General Histological Method.

Hæmatoxylin and Eosin.

a. Transfer sections from alcohol to distilled water.

b. Stain in alum-hæmatoxylin 2, 5 to 30 minutes. The stain may be prepared as follows (Boehmer):

1.	Hæmatoxylon crystals,	1 gram.
	Absolute alcohol,	10 cc.
2.	Alum,	20 grams.
	Distilled water,	200 cc.

Cover the solutions and allow them to stand over night. The next day mix them and allow the mixture to stand for one week in a wide-mouthed bottle lightly plugged with cotton. Then filter into a bottle provided with a good cork. The solution is now ready for use but its staining powers improve with age.

c. Wash the sections in several changes of water until they have lost all traces of a red tint.

d. Counter-stain with eosin ($\frac{1}{10}$ to $\frac{1}{2}$ % in 60 % alcohol) 1 to 5 minutes.

e. Alcohol, 95 %, two or three changes to dehydrate and remove excess of counter-stain.

f. Clear in oil of origanum or Dunham's mixture, white oil of thyme 4 parts, oil of cloves 1 part.

General Bacteriological Methods.

A. *Loeffler's Universal Method.*

a. Take sections out of alcohol into Loeffler's methvlen blue for 5–30 minutes.

b. Decolorize in acetic acid (0. 1%) 10 to 20 seconds.

c. Dehydrate in absolute alcohol, two or three changes, a few seconds.

d. Clear in xylene.

e. Mount in balsam.

B. *Weigert's Method.*

a. From alcohol to Ehrlich's anilin water gentian violet 5–15 minutes.

b. Wash in 0. 6% salt solution.

c. Dry with filter paper.

d. Place in potassium iodide and iodine solution (iodine 1 part, potassium iodide 2 parts, water 100 parts).

e. Dry with filter paper.

f. Decolorize in a mixture of anilin oil 2 parts and xylene 1 part, 2–5 minutes.

g. Clear in xylene.

h. Mount in balsam.

This stain can only be used with those organisms which take the Gram stain, namely: *S. pyogenes*, *M. pyogenes*, *M. aureus*, *Sar. tetragena*, *B. anthracis*, *B. pneumoniae*, *B. rhusiopathiae*, *B. tuberculosis*, *B. leprae*, *B. diphtheriae*, *P. aeruginosa*, *B. Welchii*, *B. chauvaei*, *B. oedematis*, *B. tetani and Streptothrix actinomyces.*

Special Bacteriological Methods.

Particular organisms may be stained as follows:

Pyogenic micrococci. Loeffler's or Weigert's method.

Micrococcus gonorrhoeae. Loeffler's method gives the best results.

Sarcina tetragena. Loeffler's or Weigert's method.

Bacterium anthracis. Loeffler's or Weigert's method.

Bacterium pneumoniae. Weigert's method.

Bacterium pneumonicum. The following method is recommended for staining the capsules in sections (M. & W.):

a. Stain for 24 hours in the incubator in the following solution:

Saturated alcoholic solution of gentian violet - - - -	50cc.
Distilled water - - - - - - - -	100cc.
Glacial acetic acid - - - - - - -	10cc.

b. Wash out in 1% solution of acetic acid.

c. Alcohol.

d. Xylene.

e. Canada balsam.

Bacterium cuniculicida. Loeffler's Method.

Bacterium tuberculosis.

a. Weigert's method (staining with anilin oil gentian violet 24 hours at room temperature, or 2–3 hours at 40° C.).

b. Ziehl-Neelsen's Method.

1. Stain with carbol-fuchsin (12-24 hrs. room temperature, 1-3 hrs. 40° C.)
2. Decolorize with nitric acid (10%) a few seconds and then with alcohol (60-90%) until color is nearly all extracted.
3. Counter-stain with methylen blue.
4. Dehydrate with absolute alcohol (a few seconds).
5. Clear with clove oil.
6. Xylene (and examine).
7. Mount in balsam.

Bacterium leprae. This organism is stained with the tubercle stain, unless the sections have been kept in alcohol for some time, in which case Weigert's method can be employed. To differentiate this organism from *B. tuberculosis*, stain as follows:

a. An aqueous solution of fuchsin 6–7 minutes.

b. Acid alcohol (nitric acid 1, alcohol 10) ¼ minute.

c. Wash in water.

d. Counter-stain in a saturated aqueous solution of methylen blue.

e. Alcohol.

f. Xylene.

g. Balsam.

The bacteria of leprosy stain readily by this method, tubercle bacteria do not.

Bacterium mallei.

Slow Method.

a. Stain in Loeffler's methylen blue 6–8 hours.

b. Wash in distilled water.

c. Tannic acid solution (10 %) 4–5 hours.

d. Wash thoroughly in water.
e. Dehydrate in absolute alcohol.
f. Clear in xylene and mount.

Quick Method.

a. Stain in carbol-methylen blue 10–30 seconds.
b. Wash in distilled water.
c. Tannic acid solution (10 %) ½–1 minute.
d. Counter-stain with a weak solution of eosin until sections are red.
e. Wash in water until pink.
f. Dehydrate in absolute alcohol.
g. Clear in xylene and mount.

Bacterium diphtheriae. Loeffler's or better Weigert's method.

Bacillus typhosus.

a. Loeffler's methylen blue or carbol-fuchsin 15 min.–24 hrs.
b. Wash slightly in distilled water.
c. Place in 10% solution of tannic acid for 10–60 min.
d. Dehydrate rapidly in alcohol.
e. Clear in xylene.
f. Examine.
g. Mount in balsam.

Such sections examined under a low power will be found to contain heavily stained masses, which under a high power prove to be clumps of bacilli. Not infrequently the bacilli are difficult to detect in tissue from typhoid cadavers.

Bacillus suipestifer. Loeffler's method.

Bacterium Welchii. Weigert's and Loeffler's methods.

Bacillus chauvaei. Use Pfeiffler's stain:

a. Dilute carbol-fuchsin ½ hour.
b. Absolute alcohol slightly acidutated with acetic acid until section is a reddish violet tint.
c. Xylene and examine.
d. Mount in balsam.

Bacillus oedematis. Pfeiffer's stain.

Streptothrix actinomyces.

a. Ziehl's carbol-fuchsin, 10 minutes.
b. Wash in distilled water.
c. Picric acid (cons. alc. solution).
d. Wash in distilled water.
e. Wash in alcohol (50%).
f. Dehydrate in absolute alcohol.
g. Clear in xylene.
h. Balsam.

Tissue stained yellow, rays red.

References. M. & W. 239–286; N. 537.

CHAPTER IX.

BACTERIOLOGICAL DIAGNOSIS.

EXERCISE CVI. EXAMINATION OF BUCCAL SECRETION.

DEFINITION. The secretion of the mouth, or saliva, is a mixed product derived in part from the mucous glands within the mouth and also from the parotid, submaxillary, and sublingual glands. In disease the normal character of the different parts may vary or there may be various exudates and growths present.

COLLECTION. Material for bacteriological examination is best obtained by means of a sterile probang or forceps. This material may be examined directly by means of cover-glass preparations or by means of cultures.

1. *Method of Preparing Outfit.* Wind a small piece of absorbent cotton on the end of a wire (about 1 mm. in diameter and 14 cm. long). Thrust the other end of the wire through the cotton plug of a test-tube or fasten in a cork and sterilize at 150° C. for 1 hour. This with a tube of nutrient medium (usually Leoffler's Blood serum) is placed in a box for transportation.

2. *Method of Using Outfit.* The patient is placed in a good light and the probang gently but firmly rubbed over the suspected area of the throat and then drawn gently over the surface of the medium, both tubes securely stoppered and the outfit sent to the laboratory. The organisms to be sought for are *B. diphtheriae*, the *pyogenic cocci* and *Monilia candida.*

BACTERIUM DIPHTHERIAE.

The presence of this germ in the mouth usually results in a formation of a pseudomembrane a portion of which is to be removed with a pair of forceps or by means of the outfit described above. It should, 1) be examined directly for the diphtheria bacillus by smearing on a cover-glass and staiuing by following methods:

a. Loeffler's methylen blue.

b. Gram's stain.

c. Neisser's stain: *a.* 1 gram methylen blue dissolved in 20 cc. of alcohol (96%), is added to 950 cc. of distilled water and 50 cc. of glacial acetic acid; *b.* 2 grams of bismark brown dissolved in a liter of distilled water. Films are stained in *a.* 2 to 3 seconds, washed in water, stained in *b.* 3 to 5 seconds, dried and mounted.

2) Usually, however, mere microscopical examination is not sufficient, and culture methods must be employed. In fact this method ought always to be used.

In this case make smears on Loeffler's blood serum and incubate them at 36–38° C. for 12–24 hours and then examine the growth in cover-glass preparations. The diphtheria organism if present should show:

a. Characteristic appearance with Loeffler's methylen blue.

b. Positive Neisser stain.

c. Positive Gram stain.

3) Occasionally micro-organisms (pseudo-diphtheria bacilli among others) are met with that very closely resemble the Klebs–Loeffler bacillus and render a positive diagnosis doubtful. In such cases attention to following table will be helpful:

	B. Diphtheriae	B. pseudo-diphtheriae
1) Form	Slender and of same diameter throughout	Thicker at center than ends, plumper and shorter and less variable than B. diphtheriae
2) Size	Average 1.2–2 μ	Averaging 1–1.6 μ
3) Threads	Not formed	Not formed
4) Grouping	Parallel grouping more or less characteristic but do not touch	Parallel but lie closer together
5) Involution forms	Common	Rare
6) Motility	Immotile	Immotile
7) Stains		
a. Loeffler's methylen blue	Stains readily giving banded or polar stain	Stains more regularly Polar stain rare
b. Gram	Positive	Positive
c. Neisser	Characteristic stain with very young cultures, six hours.	Not under 24 hours
8) Spores	Absent	Absent
9) Alkaline potato	Growth almost invisible	Visible and cream colored in 2 days
10) Sugar agar and gelatin stab cultures	Full length of stab	Only at upper part
11) Neutral litmus milk	Acid reaction	Alkaline reaction
12) Anaerobic cultures in H	Grows well	No growth
13) Nitroso-indol reaction	After 7 days	After 21 days
14) Inoculation experiments (Guinea pig subcutaneous)	Death 36–48 hours.	Non-pathogenic

PYOGENIC MICROCOCCI.

1) Stained cover-glass preparations are examined and if micrococci are found make:

2) Smear cultures, or better agar plate cultures and work up the colonies as they appear.

MONILIA CANDIDA (Organism of Thrush).

The material is collected by removing a portion of the patches or membrane and examining it:

1) Under the microscope in a drop of glycerine.

2) Cover-glass preparations stained with carbol-fuchsin or Gram's method.

3) By means of smear cultures on agar or blood serum, the resulting growth being examined either in glycerine mounts or stained cover-glass preparations.

REFERENCES. v. J. 95; S. 101. See also various texts under special organism.

EXERCISE CVII. EXAMINATION OF SPUTUM.

Definition. By this term is meant all of the material derived from the air passages by the act of coughing or hawking.

METHOD OF COLLECTION. For diagnostic purposes it is best collected in a salt-mouthed bottle (about 2 oz. capacity) which has been sterilized. The morning sputum is best and before being collected the mouth should be rinsed out with water.

BACTERIUM TUBERCULOSIS. Place the sputum in a Petri dish over a black surface and select one of the little cheesy masses, if these are present, and smear it on a cover-glass. Where these particles are not present a loop or two of the thick portion is used. The cover-glass preparations are to be stained by one of the following methods:

1) Gabbett, see Part 1, p. 38.

2) Ziehl-Neelson:

a. Carbol-fuchsin ten times through the flame.

b. Nitric acid (30%) momentarily.

c. Water.

d. Alcohol (60%) until red color disappears. It may be necessary to immerse preparation in acid a second time, but the greatest care must be exercised to prevent extraction of dye from tubercle bacterium.

e. Loeffler's methylen blue, 1 minute.

f. Mount and examine.

While the tubercle bacteria may be detected when present in considerable numbers with a $\frac{1}{6}$ in. objective when there are few present a $\frac{1}{12}$ in. oil immersion will be necessary, and this ought to be used to search all slides where the tubercle germ has not been found with a lower power. A mechanical stage is a great convenience in a systematic search.

At least two preparations should be stained and thoroughly examined before a negative result is pronounced.

The viscosity of sputa may be overcome and the bacteria concentrated where the number is very small by 1) Ribbert's method which consists in the addition of a 2% solution of caustic potash and boiling. This dissolves the mucus and the bacteria are then deposited with the sediment. This sediment can be obtained by allowing the mixture to stand in a conical glass vessel or more quickly by the use of a centrifuge. 2) Hammond's method:

1. Add 5% of crystallized carbolic acid (in the case of sputum add 5 times its bulk of a 5% solution of carbolic acid).

2. Place 15 cc. in the tubes of a centrifuge and whirl for 15 minutes.

3. Pour off supernatant fluid and treat precipitate with 3 cc. of a 5% KOH solution. Mix thoroughly and allow to stand 2 minutes.

4. Fill to 15 cc. mark with distilled water and whirl 20 minutes.

5. Make cover-glass preparation of sediment (or purify same by repeated washings and centrifugalizations with distilled water).

A centrifugal machine should be able to make at least 2,500 revolutions per minute. This speed ought to be maintained for 15 minutes. Sputum may be preserved by addition of small quantity of carbolic acid (5%).

Negative results are of positive diagnostic value only when repeated examinations are made of different samples taken at different times.

BACTERIUM INFLUENZAE. This micro-organism is frequently present in enormous numbers (100 or more) and sometimes in almost pure cultures in the greenish purulent masses in the sputum. It stains readily with the ordinary dyes, and when lightly stained presents the bipolar stain. Carbol-fuchsin diluted 10 times is one of the best stains. Gram's stain is negative.

Sputum from suspected cases should be collected either by means of a probang or in a bottle and examined:

1) Microscopically by staining, with a weak carbol-fuchsin, smears from the purulent masses. If a very small bacillus is in large clumps, which fails to retain stain by Gram's method, the evidence is strong that it is the influenza bacillus; the diagnosis should be confirmed, however, by

2) Cultures on blood agar.

Animal inoculations are without effect.

BACTERIUM PNEUMONIAE.

The sputum of patients suffering from pneumonia is usually of a rusty color due to presence of blood. The "pneumococcus" is readily seen in such material when stained by Gram's method, or with carbol-fuchsin and momentarily washed with alcohol, as lancet-shaped organisms with outer ends pointed and surrounded by a clear area—the capsule. The capsule can be easily stained by Welch's method. (See XXXVI.)

This organism is also frequently found in the sputum of healthy persons and small numbers may be detected by means of animal inoculation. The rabbit or mouse are most susceptible and should be inoculated intraperitoneally. As a result of infection with this organism the animal quickly dies with a typical septicaemia, the micro-organisms being found in great numbers in the blood current.

BACILLUS PESTIS. This micro-organism is frequently found in the sputum especially in the pneumonic form of the disease—for methods of detection see CX.

STREPTOTHRIX ACTINOMYCOSES. This organism has been occasionally found in sputum and in such cases the peculiar morphology of the colonies is well brought out by Gram's method. See CX.

REFERENCES. v. J. 114; S. 245. See also various texts under particular organisms.

EXERCISE CVIII. EXAMINATION OF BLOOD.

For serum test (Widal reaction) the blood may be collected and dried (see below), but in other cases where cultures are to be made the blood must be collected aseptically in sterile receptacles and hermetically sealed. For this purpose Sternberg's bulb is excellent. The skin should first be sterilized by use of corrosive sublimate or carbolic acid followed with alcohol.

It is usually well in any case to make cover-glass smears at the bed-side for microcopical examination. These are best made as follows: Place a drop of blood about the size of a pin-head on a perfectly clean cover-glass and then a second cover-glass on this; this flattens the drop of blood out into a thin film. Immediately and before coagsulation can take place the two are drawn apart horizontally and the films allowed to dry. (Cabot.)

BACTERIUM ANTHRACIS. In case of animals dead of suspected anthrax, blood or portion of spleen should be removed with least possible danger from infection or distribution of bacilli and studied as follows:

1. Microscopical examinations of blood or the spleen pulp of animals show (when stained with Loeffler's methylen blue) large bacteria in chains (5 or 6 segments) presenting the bamboo appearance.

2. In hanging drop preparation large, homogeneous, immotile bacilli.

3. Agar plate cultures should also be made and from the separate colonies subcultures; the gelatin stab being especially characteristic.

4. In important cases (as in man) guinea pigs, or white mice, should be inoculated, and in case of death organism isolated and identified.

SPIRILLUM OBERMEIERI. This organism is found in the blood only during a paroxysm. It is a long slender organism 6 or 7 times the diameter of a red blood corpuscle. (45μ) They have a brisk vibratile movement in the direction of their long axis. They are very sensitive to reagents of all kinds. Even the addition of distilled water will cause them to disappear. Fresh blood is best, but dried smears may be used and stained with fuchsin or by Gunther's method:

a. Dried films are treated with acetic acid (5%) 10 seconds, this is removed by blowing and holding film over flask of strong ammonia previously shaken.

b. Stained in Ehrlich's gentian violet.

c. Washed with water.

d. Dried.

e. Mounted in balsam or xylene.

f. Examined.

PYOGENIC MICROCOCCI. These are occasionally found and for method of detection see CX.

BACTERIUM MALLEI. Sometimes found in the blood of those suffering with Glanders. It may be detected in the blood-smears. For special methods see CX.

B. PNEUMONIAE. This germ is frequently present in fatal cases 24 to 48 hours before death. The blood should be drawn with a sterile hypodermic syringe and about 1 cc. of blood mixed with a tube of melted agar at 43°C. and poured into a Petri dish. Characteristic colonies appear in 24 to 48 hours.

B. TUBERCULOSIS. In case of miliary tuberculosis they may be very rarely found in sufficient numbers to be detected by staining methods, see sputum CVII.

B. INFLUENZAE. Canon claims to have stained and cultivated this organism in blood, but this needs confirmation.

B. COLI. This organism may be found in the blood, for methods of isolation and identification see Faeces CIX.

BACILLUS PESTIS. This germ occurs in the blood in certain cases at least but appears to require considerable skill in detecting it due to its variable appearance. Broth tubes should be infected and animals inoculated.

BACILLUS SUIPESTIFER.

a. Make agar plate and streak cultures from spleen of dead animal, and work up the colonies as they appear.

b. Widal Reaction (for technique see below under B. typhosus).

PLASMODIUM MALARIAE.

a. Examination of fresh blood. A droplet of blood from finger or lobe of ear is placed on a glass slide, covered with a cover-glass and then the cover-glass is ringed with vaselin. Examination should be made with a $\frac{1}{12}$ in. oil immersion.

b. Stained. Prepare films as directed above and stain with methylen blue and eosin or treat films with a very weak acetic acid 2 or 3 drops to 30 cc. of water; to remove haemoglobin wash with water and stain with following solution for $\frac{1}{2}$ minute:

Borax	5.0 parts.
Methylen blue	0.5 parts.
Water	100.0 parts.

Wash, dry and mount in balsam (Manson).

BACILLUS ICTEROIDES. Make agar streaks from blood or fragment of liver (where liver is obtained it is best wrapped in cloth and kept in incubator at 38°C. for 12 hours before cultures are made to encourage development of the micro-organisms, which are usually only sparingly present in tissue). Keep the cultures at 38°C. for 12-16 hours and then at 22°C. for same time; the characteristic appearance is a transparent, bluish growth surrounded by an opaque zone. If this is not obtained other cultures must be prepared and a thorough study of the organisms isolated made.

REFERENCES. v. J. 45; S. 79. See also texts under particular organisms.

WIDAL REACTION. Directions for collecting samples of blood. "Wash with boiled water the part from which the blood is to be obtained (lobe of ear, end of finger, or toe in infant). Prick deeply the skin with a clean needle." Remove two or three large drops of blood on a clean glass slide, alluminum foil, piece of isinglass or letter paper.

Allow the blood to dry. Then place in an envelope and send to laboratory and test as follows:

a. Make a hanging drop preparation from a 24–72 hour old agar, or bouillon, culture of *Bacillus typhosus.*

b. If the bacilli are actively motile, remove the cover-glass, add to the culture a small drop of a solution of typhoid blood (diluted from 10–50 times), return the cover-glass to the slide and seal well with vaselin.

c. Examine with a high dry power ($\frac{1}{6}$ in. obj.) rather than with the oil immersion.

In a typical reaction the motility is almost immediately affected and soon motion ceases altogether while the bacilli collect in clumps, i. e. become "agglutinated."

REFERENCES. v. J. 45; S. 79. See also texts under particular organism.

EXERCISE CIX. EXAMINATION OF FAECES.

The material expelled from the rectum and comprising the substances from the food and the secretions of the alimentary tract come under this head. The number of micro-organisms occuring here is enormous, and comprise a large number of species and among them several pathogenic forms particularly *B. typhosus. M. comma*, *B. tuberculosis* and *Amoeba coli.*

BACILLUS TYPHOSUS. This organism occurs in the faeces in the case of typhoid patients, but on account of the large number of other organisms its detection is very difficult The following methods are the most serviceable:

Parietti's Method. This method consists in adding Parietti's solution (carbolic acid 5 grams; hydrochloric acid 4 grams, and distilled water 100 cc.) to bouillon in the following manner: A number of tubes of bouillon have a varying quantity of the above solution added, *e. g*, 1 drop to one tube, 2 to another, 3 to another, and so on. These tubes are inoculated with a small quantity, (one or two loops), of the faeces and then placed in the 38° C. incubator. Twenty-four hours later the tube containing the largest amount of Parietti's solution which shows growth probably contains *B. coli* and *B. typhosus* if it is present. The organisms may be separated most quickly and easily by the use of the lactose litmus agar plate. The blue colonies should be worked up, and especially tested for its agglutinating power on typhoid blood. Instead of the use of the lactose litmus agar plate, either Elsner's or Hiss' methods may be used.

Elsner's Medium. Method of preparation:

Peel and cut up 500 gms. of old potatoes of medium size, add 1000 cc. of water and boil 1 and ½ hours.

Mash potatoes thoroughly; strain through a cloth and add water to filtrate to make a liter.

Add 15 % gelatin and boil 10 minutes. Cool to 60° C. and add white of one egg and boil 15 minutes.

Filter through cotton, then paper. Titrate and make gelatin 2–3 % acid. Just before tubing add 1 % potassium iodide (10 cc. of a solution in which 1 cc. contains 1 gram of potassium iodide). Tube and sterilize three times.

Plates of this medium are made in the usual way and kept at 15–18° C. On this medium the typhoid germ forms very finely granular, small, bright droplets resembling condensed moisture, while the colon bacillus gives rise to larger, brown colonies, which are more granular and spread more.

Hiss' Plate Medium. This contains·

10 grams of agar.
25 grams of gelatin.
5 grams of beef extract (Leibig).
5 grams of sodium chloride.
10 grams of glucose.
1000 grams of water.

It is made by first dissolving the agar, salt and extract in the water, then the gelatin is added and dissolved, the reaction changed by use of NaOH and phenolphthalein so that it will contain not less than 2% normal acid, cleared with two eggs and filtered, glucose added and the medium tubed and sterilized.

Make plate cultures in ordinary way and incubate at 38° C. for 18 hours, then examine the colonies microscopically. The colonies of *B. typhosus* have irregular outgrowths and fringing threads. The colonies of *B. coli*, on the other hand, are much large and as a rule are darker in color and do not form threads.

The colonies may be further examined by the use of *Hiss' Tube Medium.*

5 grams of agar-agar.
80 grams of gelatin.
5 grams beef extract (Leibig).
5 grams sodium chloride.
10 grams glucose.
1000 grams water.

Made as plate medium except that is is to contain 1.5% normal acid.

Within 18 hours at 38° C. the typhoid bacilli produce a uniform clouding. The colon bacilli do not produce uniform clouding and do produce gas.

All suspected cultures should be tested with typhoid blood (Widal reaction).

The typhoid organism may be isolated from the stools during the first two weeks of the disease.

MICROSPIRA COMMA.

1. Microscopical examination of "rice-water" discharges for spirilla lying parallel.

2. Culture methods. Gelatin or agar-plates should be made from the rice-like flakes; other flakes should be inoculated into flasks of peptone water (Dunham's solution) and inoculated at 38° C. The surface growth 6–12 hours later is to be examined microscop-

ically and by means of plates. Then test the peptone cultures for nitroso-indol (cholera red reaction) by the addition of a few drops of sulphuric acid.

BACTERIUM TUBERCULOSIS. This organism has been found in the stools in cases of intestinal ulcerations, and may come, in cases of phthisis, from ingested sputa.

AMOEBA COLI.

1. A drop of the mucous portions of stool is placed on a glass slide, covered with a cover-glass and examined with a magnification of about 500 diameters ($\frac{1}{6}$ in. objective). Examination should be conducted on a warm stage in order to get amoeboid movements.

2. Preparations may be stained with methylen blue and carmine. The nucleus is stained with the carmine.

3. Discharge may be hardened and stained by Mallory's method as follows:

a. Fix tissues in alcohol.

b. Stain (paraffin) sections in a saturated aqueous solution of thionin for 5–20 minutes.

c. Wash in water.

d. Differentiate in a 2% aqueous solution of oxalic acid ½–1 minute.

e. Wash in water.

f. Dehydrate in alcohol (95%).

g. Clear in oil of bergamot.

h. Wash with xylene and mount in balsam.

Nuclei of Amoebae brownish red, other nuclei blue.

REFERENCES. v. J. 199; Si. 228. See also texts under various organisms.

EXERCISE CX. EXAMINATION OF URINE.

For bacterial examination urine should be drawn with a sterile catheter into a sterile bottle.

BACTERIUM TUBERCULOSIS.

For method of staining see under Sputum, CVII.

It is best to centrifuge the product and care must be taken to differentiate from the Smegma bacterium. For this purpose stain cover-glass smears as follows (Bunge & Franteroth.):

1) Absolute alcohol, 3 hours.
2) Chromic acid, 15 minutes.
3) Stain in hot carbol-fuchsin.
4) Decolorize in sulphuric acid (25%) 2–3 minutes.
5) Counter-stain with a saturated alcoholic solution of methylen blue.

The smegma bacillus is decolorized by this method.

Tubercle bacterium in urine is frequently present in clusters while the smegma bacterium occurs singly. Injection of guinea pigs, smegma bacillus is non-pathogenic.

The following organisms have also been found in the urine. For methods of isolation see references.

PYROGENIC MICROCOCCI.. CXI.

M. GONORRHOEAE. CXI.

B. TYPHOSUS. CIX.

S. OBERMEIERI. CVIII.

REFERENCES. v. J. 273; Si. 504. and texts under the various organisms.

EXERCISE CXI. EXAMINATION OF TRANSUDATES AND EXUDATES.

The material should be collected in sterile vessels under aseptic precautions. Make several cover-glass preparations and stain one with Loeffler's methylen blue and the others with gentian violet or carbol-fuchsin. Mount and examine.

a. If staphylococci alone are present search for the *pyogenic micrococci.*

b. If streptococci suspect *S. pyogenes.*

c. If diplococci or tetracocci.

1. Within the pus-cells test for *M. gonorrhoeae* or *M. intracellularis.*
2. Free. *S. tetragena.*

d. If bacilli any of the following may be searched for:

1. *B. coli.* This organism is likely to be found especially in suppurative peritonitis and diseases of the urinary organs. 2. *B. anthracis.* 3. *B. pneumoniae.* 4. *B. tuberculosis.* 5. *B. leprae.* 6. *B. mallei.* 7. *B. pestis.* 8. *P. aeruginosa.* 9. *B. welchii.* 10. *B. oedematis.* 11. *B. tetani.*

e. Streptothrix actinomyces.

f. Amoeba coli.

PYOGENIC MICROCOCCI. These organisms are frequ ently present in pus and should be isolated and identified in pure cultures as microscopical examinations alone will not suffice.

STREPTOCOCCUS PYOGENES. This organism is not infrequently present and can be readily identified by culture methods.

MICROCOCCUS GONORRHOEAE. Pus should be collected in a sterile receptacle or spread on cover-glasses and allowed to dry, but should not be allowed to dry and then wet up again to spread, as this destroys the pus-cells, and hence the value of the material for diagnosis.

Stain:

1. *a.* Loeffler's methylen blue 3–5 minutes.
   b. Wash in water.
   c. Dry, mount in balsam and examine with $\frac{1}{12}$ in. oil immersion.
   d. Look for a biscuit-shaped diplococcus within the pus-cells.
2. By Gram's method.
   a. Anilin oil gentian violet 15 minutes.
   b. Wash in water.
   c. Treat with iodine solution 2 minutes.
   d. Decolorize with alcohol.
   e. Counter-stain with Bismark brown, $\frac{3}{4}$ minutes.
   f. Wash, dry and mount in balsam.
   g. Examine with oil immersion.

If the gonococci are present they will be stained brown.

If diagnosis is of great importance make cultures as follows:

1) Make 6 or more streak cultures on blood agar or better make plates on Wertheim's medium (p. 99). Grow at 38° C.

2) Make a set of ordinary agar plates or streak cultures and keep at 38° C.

The gonococcus grows on the first two media but not on the plain agar. The gonococcus is the only organism that:

1) Occurs in groups (cell-colonies) in pus-cells.
2) Is decolorized by Gram's method.
3) Does not grow on agar at room or blood heat. (Foulerton).

MICROCOCCUS INTRACELLULARIS.

Pus may be obtained by lumbar puncture which is performed as follows. The back of the patient and the operator's hands should be made sterile. The needle (4 cm. × 1 mm. for children) should be boiled 10 minutes. The patient should lie on the right side, with the knees drawn up and the uppermost shoulder so depressed as to present the spinal column to the operator. The puncture is generally made between the third and fourth lumbar vertebrae. The thumb of the left hand is pressed between the spinous processes and the point of the needle is entered about 1 cm. to the right of the median line, and on a level with the thumb nail and directed slightly upwards and inward toward the median line. At a depth of 3 or 4 cm. in children and 7 or 8 in adults the needle enters the subarachnoid space and the fluid flows usually by drops. This is allowed to drop into an absolutely clean test-tube, which has previously been plugged and sterilized. From 5 to 15 cc. of the fluid is a sufficient quantity for examination. Cultures should be made at once on blood agar and plain agar (M. & W. 371.). After standing some hours, the sediment should be examined in cover-glass preparations, stained with Loeffler's methylen blue and by Gram's method.

Micrococcus intracellularis stains by Loeffler's method and appears as a diplococcus in groups in the pus cells, is decolorized by Gram's method, and grows on blood-agar and feebly on ordinary agar at 38° C.

The following organisms are also found occasionally. For methods of diagnosis see exercises indicated.

B. COLI. CIX.

B. PNEUMONIAE. Stain for capsule. Cultivate on blood-agar. CVII.

B. TUBERCULOSIS. CVII.

B. LEPRAE. For method of staining, see CV.

B. MALLEI.

a. Widal reaction (If in man typhoid and diphtheria must be excluded in case of a positive reaction).

b. Examination of discharge.

1. Microscopical examination usually without result.
2. Cultures, glycerine agar and potato from pus.

c. Animal inoculation, Straus method.

B. PESTIS.

a. Make plate cultures from blood and buboes and work up colonies.

b. Make subcutaneous inoculation into guinea pigs from bubo, and if death ensues search for B. pestis.

P. AERUGINOSA. Easily recognized by its culture characters.

B. WELCHII.

This germ is non-pathogenic for rabbits but Welch and Flexner have shown that if a rabbit is inoculated intravenously with 0.5 to 1 cc. of a bouillon culture and killed after a lapse of 5 or 10 minutes and the animal kept at 18°–20° C. for 24 hours or at 30°–35° C. for 4 to 6 hours, the organism will multiply in the blood and produce large quantities of gas in the vessels and organs. This effect is characteristic.

B. OEDEMATIS.

a. Make cover-glass preparations from fluid of affected parts.

b. Also make anaerobic cultures. If material contains spores it should be heated to 80° C. for 10 minutes before it is seeded.

B. TETANI.

a. Make cover-glass preparation from pus and search for drumstick bacillus.

b. Make cultures in glucose bouillon and agar-plates and develop in hydrogen.

c. Inoculate animals with the discharge, and also with the bouillon culture, and watch for characteristic symptoms.

S. ACTINOMYCES.

a. Place one of the minute sulphur yellow nodules in a drop of glycerine on a glass slide and then apply gentle pressure.

b. Even the low powers of a compound microscope will then show something of the clustered arrangement which can be more carefully studied under a higher power.

c. Intraperitoneal inoculation of guinea pig. One month later nodules on peritoneum.

AMOEBA COLI. CIX.

REFERENCES. v. J. 405; Si. 514 and 518. See also texts under the various organisms.

EXERCISE CXII. DIAGNOSIS OF RABIES.

a. The medulla of the suspected animal is removed under aseptic precautions, as soon as possible after death. In case the animal is some distance from the laboratory it is best to cut off the head, pack it in ice and ship by express.

b. Place a piece of the medulla about the size of a pea, in 4 or 5 cc. of sterile bouillon and thoroughly grind up the same.

c. Anaesthetize a rabbit with ether, clip the hair from between the eyes and ears and disinfect with a carbolic acid solution.

d. Make a longitudinal incision through the skin and subcutaneous tissue along the median line, while a crucial incision is made through the periosteum on one side of median line thus avoiding haemorrhage from the longitudinal sinus. The periosteum is then pushed back and a disc of the skull ($\frac{1}{4}$ inch in diameter) removed with a trephine and the dura mater exposed.

e. With a sterile hypodermic syringe introduce 2 or 3 drops of the suspension of medulla beneath the dura mater, stitch the skin, disinfect, dry and seal the wound with collodion.

The rabbits apparently experience no inconvenience; the wound heals rapidly and the rabid symptoms appear in from 15 to 30 days, although sometimes they may occur earlier or much later.

EXERCISE CXIII. EXAMINATION OF MATERIAL FROM HUMAN AUTOPSIES.

At human autopsies smears from the organs should be made on cover-glasses and afterwards stained and examined. Plate-cultures should also be made from the various organs or instead parallel streaks over blood serum, agar-slopes or agar-plates. In all cases the surface from which the material is to be obtained should first be burned to avoid infection of cultures with extraneous germs. Portions of the various organs should also be preserved and hardened in alcohol.

CHAPTER X.

DETECTION OF PATHOGENIC BACTERIA IN WATER AND MILK SUPPLIES.

EXERCISE CXIV. EXAMINATION OF WATER FOR PATHOGENIC BACTERIA.

BACILLUS TYPHOSUS. In the examination of water it is best to concentrate the bacteria by filtering a large amount of the water through a Berkefeld filter and use the slime on the filter to make the plates.

a. Parietti's method, see CIX.

b. Hiss' method. Make plate cultures and incubate at 38° C. for 18 hours. Inoculate suspicious colonies into Hiss' tube medium, fermentation tube, milk and make indol test. Also try Widal reaction.

c. Animal Inoculation. (Michigan method).

1) Inoculate suspected water into bouillon tubes or flasks, and incubate at 38° C.

2) Twenty-four to forty-eight hours later inoculate one cc. into the peritoneal cavity of a white rat.

3) If animal recovers B. typhosus is not present. If animal dies hold autopsy and isolate and study organism causing death.

MICROSPIRA COMMA.

a. If there is reason to believe that the spirilla are very numerous gelatin plate cultures can be made directly from the water, and the suspicious colonies worked up.

b. Ordinarily the organisms are very sparse and large quantities must be used, 100-1000 cc. are placed in flasks and 1% of peptone and 0.5% salt are added, the fluid made alkaline and incubated at 38° C. for 6-24 hours. Then gelatin plate cultures are made from the upper layers and the suspicious colonies worked up as above.

EXERCISE CXV. EXAMINATION OF MILK FOR PATHOGENIC BACTERIA.

B. DIPHTHERIAE.

Where B. *diphtheriae* is suspected in milk, make a considerable number of streak cultures on Loefflers's blood serum and incubate at 38° C. for 8–12 hours and examine growth microscopically very carefully for *B. diphtheriae*.

BACTERIUM TUBERCULOSIS (Koch) Mig.

Hammond's method of examining milk for B. Tuberculosis. See Sputum, CVII.

Animal Inoculation.

Concerning the transmission of material containing Bacteria in Mails, see Postal Guide, 1898 Ruling No. 82, p. 901. Part of which is as follows: "That the order of the Postmaster General of June 1, 1893, forbidding the use of mails for the transmission of specimens of germs of cholera or other diseased tissues, is hereby modified to this extent: "Specimens of diseased tissue may be admitted to the mails for transmission to United States, State or municipal laboratories only when inclosed in mailing packages constructed in accordance with the specifications hereinafter enumerated. Upon the outside of every package shall be written or printed the words: 'Specimen for Bacteriological examination.' No package containing diseased tissue shall be delivered to any representative until a permit shall have first been issued by the Postmaster General, certifying that said institution has been found to be entitled, in accordance with the requirements of this regulation, to receive such specimens."

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