Laboratory Guide

IN ELEMENTARY BACTERIOLOGY

FROST

A LABORATORY GUIDE

IN

ELEMENTARY BACTERIOLOGY

BY

WILLIAM DODGE FROST, M. S.
INSTRUCTOR IN BACTERIOLOGY, UNIVERSITY OF WISCONSIN

ILLUSTRATED

SECOND REVISED EDITION

PUBLISHED BY THE AUTHOR
MADISON, WIS.
1902

M. J. CANTWELL, PRINTER
MADISON, WIS.

PREFACE TO SECOND EDITION.

The call for a second edition within a year seems to indicate that this Guide fills a need. The author has taken the opportunity to make changes which, he believes, will increase the usefulness of the book.

The principal change has come in the new form which will certainly be found more convenient.

The sequence of the exercises in Chap. I. has been somewhat changed and a few new subjects have been introduced. The order in which the various bacteria is taken up has been altered so that they are now to be studied in groups. This arrangement is in keeping with recent tendencies, and it is hoped that it will impress the student with the similarity between closely related forms, and also emphasize certain minute but important differences.

The addition of a number of new cuts will tend to greater clearness.

The general plan of the book remains the same.

Valuable suggestions have been received from several investigators and teachers. Especial mention is due Mr. John F. Nicholson, Assistant Bacteriologist to the Wisconsin Experiment Station, for valuable assistance.

Suggestion or criticisms will be gladly received.

WILLIAM DODGE FROST.

MADISON, WIS., JANUARY, 1902.

PREFACE TO FIRST EDITION.

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 counsel. I am also indebted to Mr. E. G. Hastings, Assistant Bacteriologist to the Wisconsin Experiment Station, for critical reading of manuscript and proof.

MADISON, WIS., January, 1902. WILLIAM DODGE FROST.

CONTENTS.

PART I.—GENERAL BACTERIOLOGY.

CHAPTER I. MORPHOLOGY AND ELEMENTARY TECHNIQUE.

CHAPTER II. PHYSIOLOGY OF BACTERIA.

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 (½ oz.) cover-glasses, 18 mm. (¾ 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.).
6 fermentation tubes.
6 glass tumblers or tin cans.
2 small wire baskets.
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 potato knife.
1 Bunsen burner with tubing.
1 piece of wire gauze.
1 tripod with reducing rings.
1 rice cooker.
3 large 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. 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.

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

III. 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 aud water.

IV. 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.

V. 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.

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

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

VIII. 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.

IX. 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.

X. 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.

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 Bacterilogical Technique. Oliver & Boyd, London, 1899.
C.—	Chester: A Manual of Determinative Bacteriology. The MacMillan Co., New York, 1901.
Cn.—	Conn: Agricultural Bacteriology. Blakiston's Son & Co., Philadelphia, 1901.
Co.—	Connell: A Laboratory Guide in Practical Bacteriology. Author, Kingston, Ontario, 1899.
Cu.—	Curtis: Essentials of Practical Bacteriology. Longmans, Green & Co., New York, 1900.
F.—	Fischer: Structure and Functions of Bacteria. Clarendon Press, New York, 1900.
Fl.—	Fluegge: Die Mikro-organismen, F. C. W. Vogel, Leipzig, 1896.
Fr.—	Frankland: Micro-organisms of Water. Longmans, Green & Co., 1894.
G.—	Gage: The Microscope. Comstock Pub. Co., Ithaca, N. Y., 8th Edit., 1901.
Go.—	Gorham: Laboratory Course in Bacteriology, W. B. Saunders & Co., Philadelphia, 1901.
H.—	Hewlett: Manual of Bacteriology. Blakiston's 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. McMillan Co., New York, 1895.
L.—	Lafar: Technical Mycology. Vol I. Lippencott Co., Philadelphia, 1898.
L. & K.	Levy & Klemperer: Clinical Bacteriology. Saunders & Co., Philadelphia, 1900.
L. & N.—	Lehmann & Neumann: Atlas and Essentials of Bacteriology. W. B. Saunders & Co., Philadelphia, 1901.
M.—	Moore: Laboratory Directions for Beginners in Bacteriology. Ginn & Co., New York, 1900.
Mig.—	Migula; System der Bakterien, Gustav Fischer, Zena, 1900.
M. & R.—	Muir & Ritchie: Manual of Bacteriology. MacMillan Co., New York, 2nd Edit., 1899.
M. & W.—	Mallory & Wright: Pathological Technique. W. B. Saunders & Co., Philadelphia, 1897.
McF.—	McFarland: Text-Book of Pathogenic Bacteria. W. B. Saunders & Co., Philadelphia, 3rd Edit., 1900.
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. Bailliére, 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. P. Blakiston's Son & Co., Philadelphia, 1898.

PART I.

GENERAL BACTERIOLOGY.

PART I.—GENERAL BACTERIOLOGY.

CHAPTER I.

MORPHOLOGY AND ELEMENTARY TECHNIQUE.

EXERICSE 1. 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 running 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 culture, 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. Read Rule I. Clean as directed above, all flasks, test-tubes, fermentation tubes and Petri dishes in your possession.

EXERCISE 2. 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 non-absorbent and of the best 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. 164; P. 223.

SPECIAL DIRECTIONS. Plug all test-tubes, flasks and fermentation tubes in your possession.

EXERCISE 3. 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. 164; 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 4. 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 (or buy Hamburg steak).	*a.* Ditto.	*a.* Weigh out three grams of beef extract (such as Liebig's).
b. Add 1 liter of distilled water and after thoroughly shaking set in ice chest for 12 to 24 hours.	*b.* Add 1 liter of distilled water.	*b.* Add 1 liter of water.
c. Squeeze through a cloth and add enough distilled water to filtrate to make 1 liter and place in vessel. This may be either a flask which is heated in a water-bath or sterilizer, or a rice-cooker. In case a rice-cooker is used a 50% solution of calcium chloride should be placed in the 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.	*c.* Place in vessel for cooking, weigh vessel and contents so that water driven off in cooking may be accurately replaced, then cook for ½ hour at about 70° C., filter through paper.	*c.* Place in vessel for cooking.

d. 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.

e. Heat, not above 60° C., until ingredients are in solution, then restore the water lost by evaporation.

f. Neutralize. *This is a very important step and calls for great care.* Of the following methods, A is more accurate and should be employed for special or research work. For ordinary routine work B may be employed.

A.	B.
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%	Use a normal[1] solution of sodium hydroxide ($\frac{n}{1}$Na OH). Add to the hot solution a few cc. at a time at first, later a few drops, stirring thoroughly

[1] 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.

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.

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 phenolphthalein paper. (Prepared by dipping filter paper in a solution). The addition should continue until the test paper is turned a faint pink color.

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.

g. Boil for 5 minutes and restore weight.

h. Test reaction and adjust if necessary.

i. Add 0.5 to 1.5% of a normal hydrochloric acid. The amount of acid to be added varies with the purpose for which the medium is to be used, e. g., in water analyses + 1.0 (acid) is preferable, with the pathogenic bacteria a smaller amount of acid (+ 0.5) more nearly meets requirements.

j. Heat until precipitate appears flaky and then filter through moistened filter paper. (For method of folding see Abbott p. 96).

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. 187; 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 **7**. See Rule IV.

EXERCISE 5. 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 vessel 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 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.

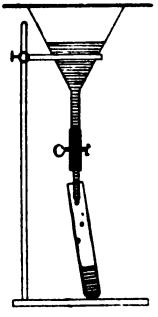


FIG. 1. Apparatus for filling test-tubes.

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

EXERCISE 6. STERILIZATION OF CULTURE MEDIA.

EXPLANATORY. To sterilize culture media 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. A simple steam sterilizer is shown in Fig. 2, and for student use is very convenient. The method of using either form is identical. Always have plenty of water present before heating. 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 spore germination, i. e., at the room temperature. This method of sterilization is known as the discontinuous method or Tyndallization.

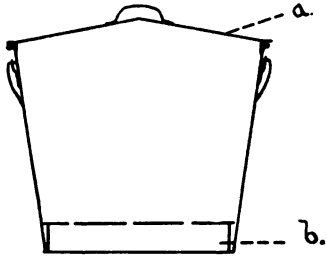


FIG. 2. Simple sterilizer consisting of a galvanized iron pail with a cover *a* and a false bottom *b*.

For the employment of steam under pressure the autoclav 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 indicated on the guage:

Atmospheres.	*Degrees C.*
0.0	100
0.5	112.2
1.0	**121.4**
1.5	128.8
2.0	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. In case of gelatin and sugar media the temperature should not exceed 110° for 15 minutes.

REFERENCES. A. 55–73; M. & R. 37; McF. 166; N. 161; P. 213.

SPECIAL DIRECTIONS. Sterilize bouillon prepared in **4** for 20 minutes in a steam sterilizer on three consecutive days, or in the autoclav at 120° C. for 20 minutes. Rule V.

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.

EXERCISE 7. PREPARATION OF GELATIN.

GENERAL DIRECTIONS.

a.
b. } Same as bouillon (**4**).
c.

d. Add 1% peptone, 0.5% salt and 10–15%[1] of the best gold label, sheet gelatin, and weigh.

e. Heat until ingredients are dissolved.

f. Neutralize.

g. Boil 5 minutes and restore weight.

h. Test reaction.

[1] 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.

i. Boil until albumin coagulates and floats in the clear fluid. If beef extract is used it will be necessary to first cool below 60° C. and thoroughly stir in an egg.

j. Filter. Arrange the apparatus shown in Fig. 3. Use absorbent cotton. The funnel and flask should first be heated with warm water. Usually the hot gelatin will filter without the use of the pump. If the pump is needed it should be started before pouring in the culture medium. This prevents the unfiltered gelatin from passing between the cotton and glass.

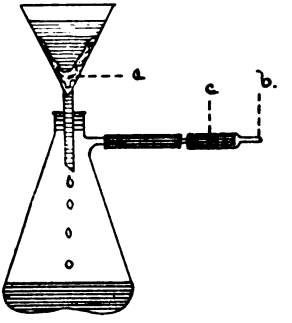


Fig. 3. Apparatus for filtering media through absorbent cotton; *a*, layer of cotton; *b*, tubes for making connection with air pump; *c*, Bunsen valve to prevent entrance of water into flasks.

k. Add 5.0 cc. of a normal hydrochloric acid.

l. Tube. (**5.**)

m. Sterilize in the steamers for 30 minutes on three consecutive days or in the autoclav at 110° C. for 15 minutes.

REFERENCES. A. 95; H. 42; M. & R. 46; McF. 190; 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 autoclav. Remember *long exposure to high heat* injures the solidifying properties of gelatin.

EXERCISE 8. PREPARATION OF AGAR.

GENERAL DIRECTIONS.

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

a. Same as *a* in the preparation of bouillon (**4**).

b. Add 500 cc. of distilled water.

c. Same as bouillon (**4**).

d. Add 20 gms. of peptone and 5 gms. of salt.

e. Heat until peptone is dissolved.

f. Neutralize.

g. Cool to 60° C., add agar solution and mix (in case extract is used it will be necessary to add an egg at this point).

h. Boil until albumin is coagulated and floats in the clear liquid and restore weight.

i. Test reaction.

j. Add 0.5% acid.

k. Filter as in case of gelatin. (**7** *j.*)

l. Tube.

m. Sterilize in steam for 15 minutes on three successive days or in autoclav 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 solidfied in an upright position can be used for plate cultures.

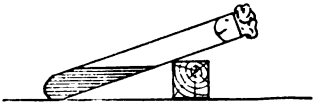


FIG. 4. Method of sloping agar.

REFERENCES. A. 100; H. 43; M. &. R. 48; McF. 191; 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 9. 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.

FIG. 5. Bolton's potato tube.

Unless the tubes are to be used immediately, they should be sealed. (**11.**) The dark color can be prevented by immersing the pieces between *c* and *d* in running water for from 12–18 hours.

REFERENCES. A. 104; M. & R. 54; McF. 194; N. 183; P. 216; P. B. C. 28; S. 47.

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

EXERCISE 10. 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 autoclav 15 minutes at 120° C., or in steamer 15 minutes on three successive days.

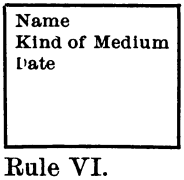
SPECIAL DIRECTIONS. Prepare and sterilize 10 water-blanks.

EXERCISE 11. 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 tube-tests 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, or rubber dam easily obtained from dentists and fastened on with a rubber band may also be used; 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.

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, leaving the rest of the label to be filled in when the medium is inoculated.



Rule VI.

EXERCISE 12. 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.

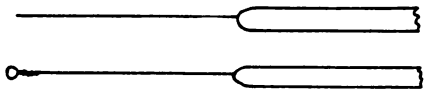


FIG. 6. Platinum Needles.

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

EXERCISE 13. 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 imposible 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 from the air.

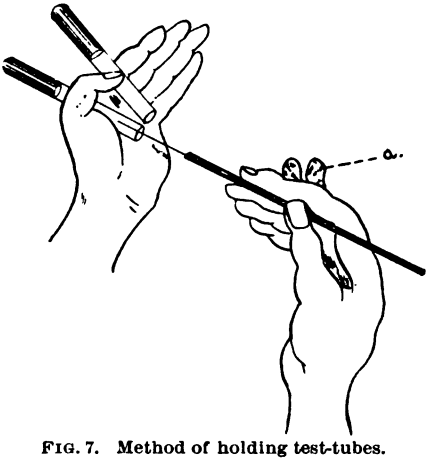


FIG. 7. Method of holding test-tubes.

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. 209.

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. Rule VIII.

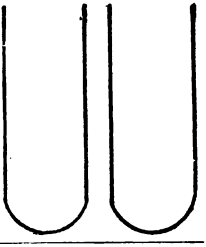
b. Label *each tube*, writing the name of the *organism*, the *date* of inoculation and *your own name*. Rule VI.

c. Place the gelatin in the cool chamber, and the other cultures in the incubator at 28° C. See next Exercise.

EXERCISE 14. 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 appara-

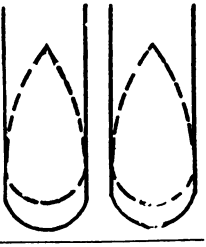
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

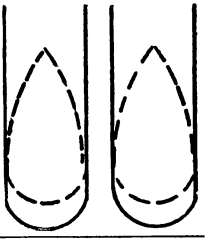
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

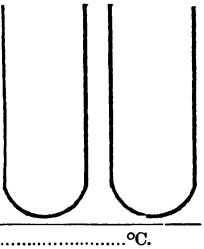
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

tus 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 15. STUDY OF TEST-TUBE CULTURES.

GENERAL DIRECTIONS. As soon as growth becomes visable 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.

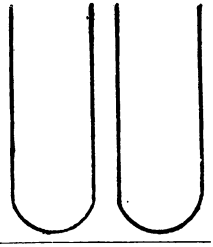
For solid cultures (agar and potato slopes), note: 1) Form of growth, 2) size, 3) surface elevation, 4) consistency, 5) color, 6) effect on media, and 7) characteristic odor.

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.

REFERENCE. C. 19; P. B. C. (Charts by Cheesman.)

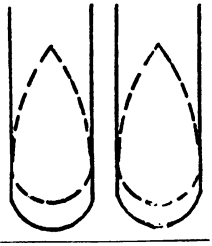
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

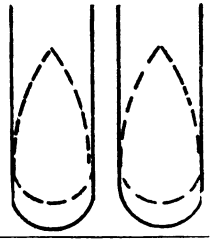
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

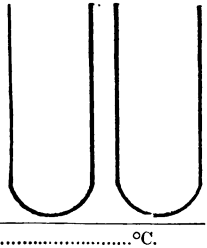
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

SPECIAL DIRECTIONS. Study, write careful descriptions and make necessary drawings of all the cultures made. For recording results use the table on pages 23 and 25.

EXERCISE 16. 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 by rubbing them between driers (two wooden 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 17. 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 Bismarck brown. They keep 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 Bismarck 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 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. & R. 103; McF. 153; 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.

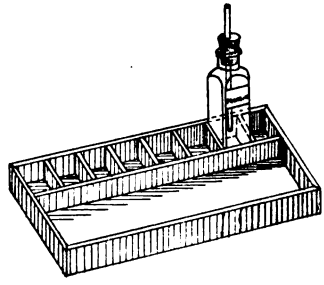


Fig 8. Block for stain bottles.

EXERCISE 18. SIMPLE COVER-GLASS PREPARATION.

GENERAL DIRECTIONS. Bacteria may be studied under the microscope in a living condition in a hanging drop preparation (**21**); 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 next exercise).

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.

Resumé.

a. Spread film,
b. Air dry,
c. Fix,
d. Stain,

e. Mount in water,		*e.* Dry,
f. Examine,	or	*f.* Mount in balsam,
g. Dry and mount in balsam.		*g.* Examine.

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

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

SPECIAL DIRECTIONS.

a. Make cover-glass preparation from agar streak of *B. subtilis* (**13**) staining with an aqueous solution of gentian violet for 5 minutes.

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 19. 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 oculars, 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 ocular 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; N. 123; P. 206.

SPECIAL DIRECTIONS. Examine cover-glass preparations made in previous exercise, first with $\frac{1}{6}$ in. objective, and then with the oil-immersion objective. If the specimen be satisfactory, sketch as directed in next exercise.

EXERCISE 20. 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 the result be not in terms of the micron (μ) it should be converted to such, as this is the unit in micrometry.

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. ($\frac{3}{4}$ mm.)		
Oil-immersion.		

b. Measure the bacteria on the preparations made in exercise 18 and sketch a few individuals from each.

In making drawing, represent a micron by two and one-half millimeters on paper. This will give a magnification of 2,500 diameters, represented thus: ×2,000.

EXERCISE 21. HANGING-DROP PREPARATION.

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.

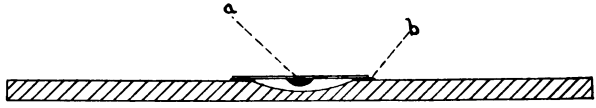


FIG. 9. Hanging-drop preparation. *a.* Hanging drop; *b.* Vaselin.

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. 144; N. 142: P. 209.

SPECIAL DIRECTIONS.

a. Make a hanging-drop preparation of water containing particles of India ink or carmine in suspension. This illustrates molecular or Brownian movement.

b. Make a preparation using straw infusion or tartar from teeth to note variations in rate and character of vital movement.

c. Make hanging-drop preparation of *B. subtilis* from agar or bouillon (13).

d. Make same preparation of *B. coli* (13).

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 22. MICROSCOPICAL STUDY OF FORM TYPES.

a. Make bouillon and agar streak cultures 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 cultures at 28° C. for 24 hours.

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

d. Examine with the oil-immersion objective, and write the names of the organisms in their proper places in the table below:

Shape of organism.	Relative size.	Name.	Sketch.
Spherical.	Medium.		
	Small.		
Elongated.	Large.		
	Small.		
Spiral.	Short.		
	Long.		

e. Make sketches of each organism.

f. Mount all preparations in balsam and hand them to instructor for inspection.

EXERCISE 23. STUDY OF CELL GROUPING.

IMPRESSION PREPARATIONS. The exact relation of cell to cell as they develop in the colony can frequently be determined best 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* 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 arrangements of adherent bacteria are 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.

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 places, as determined by cell grouping, in the following scheme:

Arrangement.	Form.	Name.	Sketch.
Isolated.	Spheres.		
	Rods.		
	Spirals.		
Filaments.	Spheres.		
	Rods.		
	Spirals.		
Plane surfaces.	Spheres.		
Regular masses.	Spheres.		
Irregular masses.	Spheres.		
	Rods.		

AGAR HANGING-DROP CULTURES (Wesbrook).

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 24. 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 25. STUDY OF ENDOSPORES.

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

Bacillus subtilis (EHRENB.) COHN.

Bacterium anthracis (KOCH) MIG. (or *Bacillus mycoides* FLUEGGE).

Bacillas amylobacter VAN TIEGHEM (or any clostridium form).

Bacillus tetani NICOLAIER (or any "drumstick" bacillus).

b. When the cultures are 48 hours old mount films without staining, examine and fill out following table:

Size of Spore.	Position.	Name of organism.	Sketch.
Smaller than diameter of mother-cell.	Median.		
	Polar.		
Larger than diameter of mother-cell.	Medium.		
	Polar.		

c. Simple stain.

1. Prepare film of *B. subtilis.*

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

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

4. Mount and examine.

d. Double stain (Hauser's method).

1. Prepare a film of any of the above organisms providing a previous examination has shown that the spores are fully developed and the mother-cells have not disintegrated.

2. Fix, three times throught the flame.

3. Stain with hot (steaming) carbol-fuchsin for 5 minutes.

4. Cautiously decolorize with 5 per cent. acetic acid until the pink color is nearly removed from the film.

5. Wash thoroughly in water.

6. Dry (blot).

7. Stain with Loeffler's methylen blue, 3 minutes.

8. Mount and examine. The spores should appear crimson in blue bacilli.

REFERENCES. A. 164–167; J. H. 26; L. 60; L. & N. 25; M. & R. 6; McF. 156; N. 46; P. 46 & 203; P. B. C. 15; S. 114.

EXERCISE 26. 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 (**16**). Allow this 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 while it is held in the hand, (not in the forceps, as over heating will injure the preparation) through the top of the flame.

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. 158; P. 205. Other methods A. 167; M. & R. 115.

SPECIAL DIRECTIONS. Stain *B. typhosus* from cultures furnished, also try *B. coli* and *B. subtilis.*

EXERCISE 27. 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. Apply Ziehl's carbol fuchsin 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, blood of rabbit infected with the *Bact. pneumoniæ* or a milk culture of a capsule bearing organism as *Bact. pneumonicum* (Fried.) Mig. or *Bact. capsulatum* (Stern.) Chester.

EXERCISE 28. STAIN FOR METACHROMATIC GRANULES (ERNST).

a. Stain a young culture of an organism such as *Bact. diphtheriæ* with Loeffler's methylen blue for about 3 minutes.

b. Wash in water.

c. Treat with a saturated solution of Bismarck brown for 30 seconds.

d. Wash in water, mount in water and examine, or, dry, mount in balsam and then examine.

The granules should appear blue in a brown organism.

EXERCISE 29. MORPHOLOGY OF YEASTS AND MOULDS COMPARED WITH BACTERIA.

a. Mount some baker's yeast (*Saccharomyces cerevisiæ*) and examine in an unstained condition. Compare: Size; form; structure and method of reproduction with the bacteria.

b. In same way examine a number of common moulds, e. g. *Mucor, Penicillium and Aspergillus.*

EXERCISE 30. GELATIN PLATE CULTURES.

EXPANATORY. 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 and spread out on a horizontal surface to cool. The dilution is such that the individuals are separated from each other by several millimeters. In the solidified medium the organisms are fixed and their growths result 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. 10). 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 be 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 two 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. 11), 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

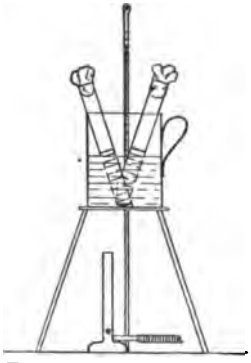


FIG. 10. Method of melting gelatin.

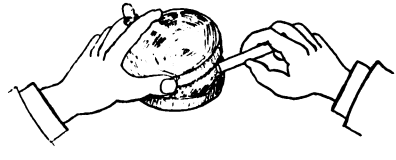


FIG. 11. Method of pouring plates.

harden by placing the dish on the cooling apparatus, or leaving it on a 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. 12). 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 will take 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 appearances, in varying numbers, depending upon the dilution used.

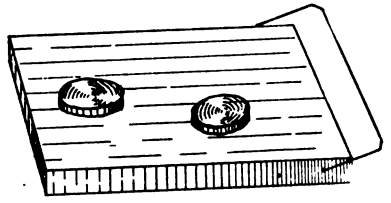


FIG. 12. Soapstone used for solidifying gelatin in Petri dishes.

Inasmuch as the first plate is generally 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 used simply to dilute the material.

REFERENCES. A. 124; H. 57; L. & K. 88; M. & W. 108; M. & R. 61; McF. 203; 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 (**13**) 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 (**14**).

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 will indicate carelessness.

EXERCISE 31. 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 45° 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. 13. 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 onto 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.

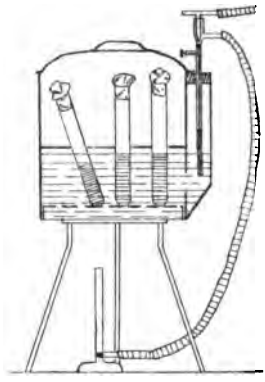


Fig. 13. Water-bath for cooling agar.

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 (**13**) for tube No. 1 and proceed as in **30.** *b.* Invert and place in incubator at 28° C.

EXERCISE 32. 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. 14). 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 successfully 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

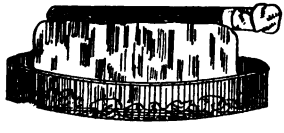


FIG. 14. Method of Making Roll-Cultures.

attached to the tube; afterwards they can be stored in the usual receptacles for tube cultures.

REFERENCES. A. 131; M. & R. 65; McF. 206.

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* (13), 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 33. STUDY OF PLATE CULTURES.

MICROSCOPIC. 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.).

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.)

REFERENCES. C. 20; 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 34. 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 decolorizing agents.

EXERCISE 35. 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 1½ minutes.
d. Pour off stain and without washing.
e. Apply iodine 1½ minutes.
f. Apply 96% alcohol 3 minutes, or 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. 152; N. 287; P. 203.

SPECIAL DIRECTIONS. Stain films of young cultures of *B. coli.* and *B. subtilis.*

EXERCISE 36. TUBERCLE STAIN (GABBETT.)

EXPLANATORY. All of the differential methods of staining the tubercle bacterium depend upon the fact that this germ is very resistent 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 ½ to 1 minute.
f. Wash in water and examine.
g. Dry and mount in balsam.

REFERENCES. A. 162; M. & W. 92; McF. 295; P. 304.

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

CHAPTER II.

PHYSIOLOGY OF BACTERIA.

EXERCISE 37. PREPARATION OF SPECIAL MEDIA.

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

a. DEXTROSE BOUILLON. To ordinary bouillon add 1% dextrose (C. P.), tube and sterilize in *steamer*, not in autoclave, 7 test-tubes and 2 fermentation tubes.

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

c. DEXTROSE AGAR. 1% dextrose (C. P.), tube and sterilize in steamer, 5 tubes.

d. LACTOSE AGAR. 1% lactose (C. P.), tube and sterilize in steamer, 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. DEXTROSE-FREE BROTH. 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. (Between *b.* and *c.* Exercise 4.) The bouillon is then prepared in the usual manner.

Or DUNHAM'S SOLUTION.

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

g. NITRATE SOLUTION.

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

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. for **41.** and **44.**) 10 tubes of gelatin, 15 tubes of agar, 6 water-blanks and 5 potato tubes.

(If thought desirable the media required for chapters IV. and V. (Exercise **58**), may be prepared at this time; this would then complete all the media making required in Part I.)

EXERCISE 38. 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. 35; McF. 46.

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

EXERCISE 39. 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 40. 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. 44.

SPECIAL DIRECTIONS. Use a mesophilic bacterium as *B. coli* and a psychrophilic organism as *Ps. violacea.*

EXERCISE 41. 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, 6 test-tubes (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 one of the tubes, inoculate the broth with three loopfuls (standard size, 12) of the 48 hour old culture (*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 immediately in a vessel of ice cold water to cool. Then incubate at a temperature favorable to the development of the organism under observation.

e. Raise the temperature of the bath 5 degrees, *i. e.* to 50° C. inoculate another tube. Keep it at 50° for 10 minutes, remove, cool and incubate.

f. In the same manner expose the organism to the following temperatures: 55°, 60°, and 65° C. for a period of 10 minutes each.

g. 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.)

REFERENCE. P. B. C. 32.

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

EXERCISE 42. 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 43. EFFECT OF DESICCATION.

GENERAL DIRECTIONS.

a. Prepare five cover-glasses each of a spore-bearing and a non-spore-bearing culture, as directed in **42.**

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. 40; 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 44. 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 does not prevent growth in No. 1 or its sub-culture. 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. 37; L. 107; McF. 49.

SPECIAL DIRECTIONS. Use *B. coli.*

EXERCISE 45. 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. 41; L. 180; McF. Chap. VIII.

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

EXERCISE 46. 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. 46; L. 77; McF. 46; S. 151.

SPECIAL DIRECTIONS. Use *B. prodigiosus* (Ehrenb.) Fluegge. Sketch.

EXERCISE 47. DETECTION OF GAS (SHAKE CULTURE).

GENERAL DIRECTIONS.

a. Melt a tube of dextrose 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. 89; M. & R., 85.

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

EXERCISE 48. 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 Frost's gasometer. (Plate I.)

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 remaining 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. 56; M. & R. 86.

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

EXERCISE 49. DETECTION OF ACIDS AND ALKALIES (WURTZ).

GENERAL DIRECTIONS.

a. Melt a tube of lactose agar (or lactose gelatin) and add enough of a sterile, blue litmus solution (**37** *e.*) 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.

REFERENCE. McF. 55.

SPECIAL DIRECTIONS. Use a sewage putting a drop in a water blank and using a loop or two of this.

EXERCISE 50. QUANTITATIVE DETERMINATION OF ACIDS.

GENERAL DIRECTIONS.

a. Inoculate 5 test-tubes of dextrose 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 51. DETECTION OF NITRITES IN CULTURES.

GENERAL DIRECTIONS.

a. Make a culture of a reducing organism in a test-tube of the nitrate solution (**37** 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) *a*-amido-naphthalene acetate. Boil 0.1 gram of solid *a*-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. (Leffmann 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. 57.

SPECIAL DIRECTIONS. Use sewage.

EXERCISE 52. DETECTION OF AMMONIA..

GENERAL DIRECTIONS.

a. Make bouillon culture and incubate 24-48 hours later:

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.

REFERENCE. L. & N. 78.

SPECIAL DIRECTIONS. Use sewage to inoculate medium.

EXERCISE 53. DETECTION OF SULPHURETTED HYDROGEN.

GENERAL DIRECTIONS.

a. Make a culture in a test-tube, or better, in 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.

REFERENCE. L. & N. 76.

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

EXERCISE 54. DETECTION OF INDOL.

GENERAL DIRECTIONS.

a. Make two cultures in tubes of sugar-free broth (or Dunham's solution).

b. Five days later add a few drops of concentrated sulphuric acid. The appearance of a pink color indicates that *nitroso-indol* has been formed, (cholera-red reaction). If the pink or deep red color does not appear, add 1 cc. of sodium nitrite solution, (sodium nitrite 0.02 grams and distilled water 100 cc). The appearance of a red color indicates formation of *indol.*

REFERENCES. L. & N. 142; McF. 57; M & R. 87.

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

EXERCISE 55. DETERMINATION OF CHEMICAL ENZYMS 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 and then add to each $\frac{1}{10}$ cc. of a 5% solution of carbolic acid for each cc. of medium, shake thoroughly and filter.

b. Pour one into a tube of sterile gelatin and the other into a tube of milk and note changes.

REFERENCE. McF. 61.

SPECIAL DIRECTIONS. Use *B. subtilis* or *B. prodigiosus.*

EXERCISE 56. VARIATION IN ENZYME PRODUCTION.

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

EXERCISE 57. 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.

In order to become acquainted with a particular organism, to differentiate it from its congeners or to assign it a definite place in a system of classification, it must be studied under various conditions and its characters determined as indicated in the following table.

POINTS TO BE OBSERVED IN THE STUDY OF BACTERIA.

The following scheme gives the most important points to be noted in the description of an organism together with some of the more common descriptive terms suggested by Chester and others.

MORPHOLOGICAL CHARACTERS.

a. Form and arrangement: *Spherical, micrococcus,* single and irregularly grouped; *diplococcus, streptococcus, tetracoccus, sarcina, rods,* single, in chains and in filaments; *spirals.*

b. Size.

1. In terms of the micromillimeter; breadth, average and extreme length.

2. In terms of human blood cells.

c. Stain.

1. Aqueous solutions; stains easily or with difficulty; uniformly or irregularly.

2. Special stain; Gram; tubercle; etc.

d. 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, monotrichous, lophotrichous or peritrichous.

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

f. 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.

g. Vacuoles (plasmolysis).

h. Crystals.

i. Involution forms.

j. Pleomorphism.

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

CULTURE CHARACTERS.

PLATE-CULTURES (Gelatin and Agar).

I. *Surface Colonies.*

1. Form: *Punctiform*, too small to be defined by the naked eye; *circular; oval; fusiform*, spindle-shaped, tapering at each end; *cochleate*, twisted like a snail shell (Fig. 15, A); *conglomerate*, an aggregate of similar colonies (Fig. 15, B); *ameboid*, very irregular like the changing forms of amebae (Fig. 15, C); *rhizoid*, of an irregular branched root-like character (Fig. 15, D); *floccose*, of a dense woolly structure; curled, filaments in parallel strands, like locks or imilets (Fig. 15, E); *myceloid*, a filamentous colony with the radiate character of a mould (Fig. 15, F); *filamentous*, an irregular mass of loosely woven filaments (Fig. 15, G); *rosulate*, shaped like a rosette.

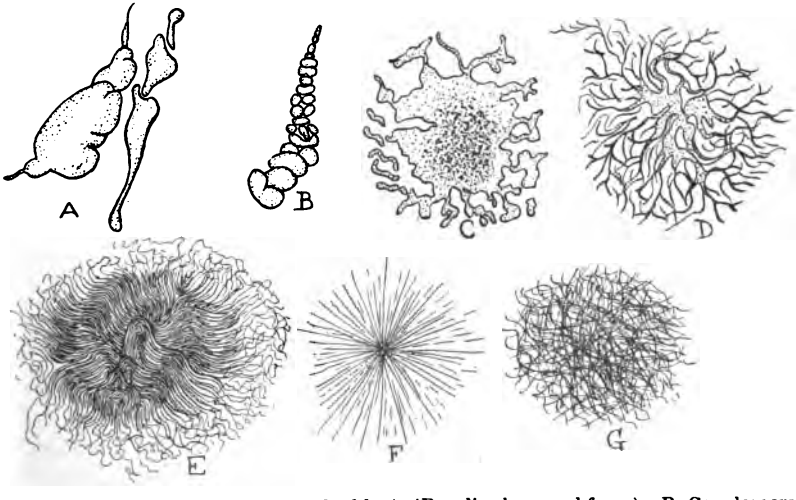


FIG. 15. Types of colonies. A. Cochleate (B. coli, abnormal form). B. Conglomerate (B. Zopfii). C. Ameboid (B. vulgatus). D. Rhizoid (B. mycoides). E. Curled (B. anhracis). F. Myceloid (B. radiatus). G. Filamentous.

2. Size expressed in millimeters.

3. Surface elevation: *Flat*, thin speading over the surface (Fig. 16, a); *effused*, speading over the surface as a thin veilly layer, more delicate than the preceeding; *raised*, thick growth, with abrupt, terraced edges (Fig. 16, b); *convex*, surface segment of a circle, but very flatly convex (Fig. 16, c); *pulvinate*, surface segment of a circle but decidedly convex (Fig. 16, d); *capitate*, hemispherical (Fig. 16, e); *umbilicate*, shaped like a navel (Fig. 16, f); *umbonate*, bearing a knob in the center (Fig. 16, g).

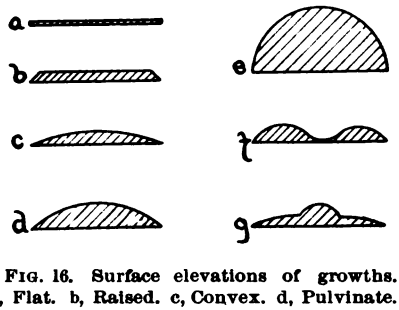


FIG. 16. Surface elevations of growths. a, Flat. b, Raised. c, Convex. d, Pulvinate. e, Capitate. f, Umbilicate. g, Umbonate.

4. Topography of surface: *Smooth*, surface even without any of the following distinctive characters; *alveolate*, marked by depressions separated by thin walls so as to resemble a honey comb; *punctate*, dotted with punctures like pin-pricks; *bullate*, like a blistered surface, rising in convex prominences, rather coarse; *vesicular*, more or less covered with minute vesicles due to gas formation, more minute than bullate; *verrucose*, wart-like, bearing wart-like prominences; *squamose*, covered with scales; *echinate*, beset with pointed prominences; *papillate*, beset with nipple or mamma like processes; *rugose*, short, irregular folds due to shrinkage; *contoured*, an irregular but smoothly undulating surface like the surface of a relief map; *rimmose*, abounding in chinks, clefts, or cracks.

5. Microscopic structure.

A. Colony as whole: *Power of refraction*, weak or strong; *amorphous*, without definite structure; *hyaline*, colorless or clear; *homogenous*, structure uniform throughout; *areolate*, divided into rather irregular or angular spaces by more or less definite boundaries (Fig. 17, 1); *granular*, finely or coarsely; *grumose*, clotted appearance, particles in clustered grains (Fig. 17, 2); *moruloid*, having the character of a morula divided into more or less regular segments. (Fig. 17, 3); *clouded*, having a pale ground with ill-defined patches of deeper tint (Fig. 17, 4); *gyrose*, marked by wavy lines indefinitely placed (Fig. 17, 5); *rivulose*, marked by lines like the rivers of a map; *rimmose*, showing chinks, cracks or clefts; *marmorated*, showing faint, irregular stripes, or traversed by vein-like markings as in marble (Fig. 17, 6); *reticulated*, in the

form of a network, like the vein of a leaf (Fig. 17, 7); *filamentous, floccose,* or *curled,* as defined under *a.* above.

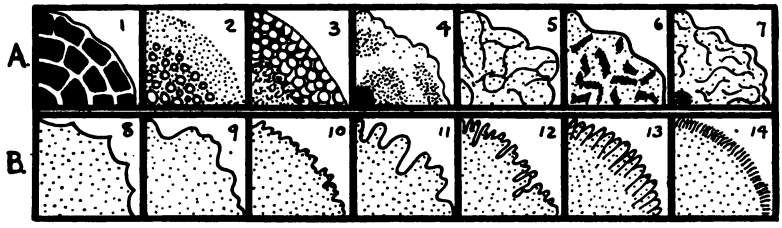


FIG. 17. Microscopic structure of colonies. A, Colony as a whole. B, Edge of colony. 1, Areolate; 2, Grumose; 3, Moruloid; 4, Clouded; 5, Gyrose; 6, Marmorated; 7, Reticulate; 8, Repand; 9, Lobate; 10, Erose; 11, Auriculate; 12, Lacerate; 13, Fimbricate; 14, Ciliate.

B. Edge of colonies: *entire,* without toothing or division; *undulate,* wavy; *repand,* like the border of an open umbrella (Fig. 17, 8); *lobate,* (Fig. 17, 9); *erose,* as if gnawed, irregularly toothed (Fig. 17, 10); *auriculate,* with ear-like lobes (Fig. 17, 11); *lacerate,* irregularly cleft, as if torn (Fig. 17, 12); *fimbricate,* fringed (Fig. 17, 13); *ciliate,* hair-like extensions, radially placed (Fig. 17, 14); *filamentous,* (Fig. 15, G); *curled,* (Fig. 15, E).

6. Color (To be determined for both transmitted and reflected light): *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, porcelanous; *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 and yellow; *ceraceous,* translucent and wax colored; *opaque; cretaceous,* opaque and white; *chalky,* dull without lustre; *glossy,* shining; *fluorescent; iridescent.*

7. Consistency: *hard, friable; soft; viscid.* Changes in medium: *Liquefaction* (gelatin), shape of liquified area, character of the fluid, membrane and sediment see under Bouillon below; *color; odor; consistency.*

II. *Deep colonies.*

1. Form. 2. Size. 3. Character of surface. 4. Microscopic structure. 5. Consistency. 6. Changes in medium. Same as surface colonies.

STAB CULTURES (Gelatin or Agar).

I. *Non-liquefying.*

1. Line of puncture: *filiform*, uniform growth without any special characters (Fig. 18, 1); *nodose*, consisting of closely aggregated colonies; *beaded*, loosely placed or disjointed colonies (Fig. 18, 2); *papillate*, covered with papillae; *echinulate*, minutely prickly (Fig. 18, 3); *villous*, beset with undivided hair-like extensions (Fig. 18, 4); *plumose*, a delicate feathery growth; *arborescent*, beset with branched hair-like extensions (Fig. 18, 5).

2. Surface growth. Same as for plate cultures.

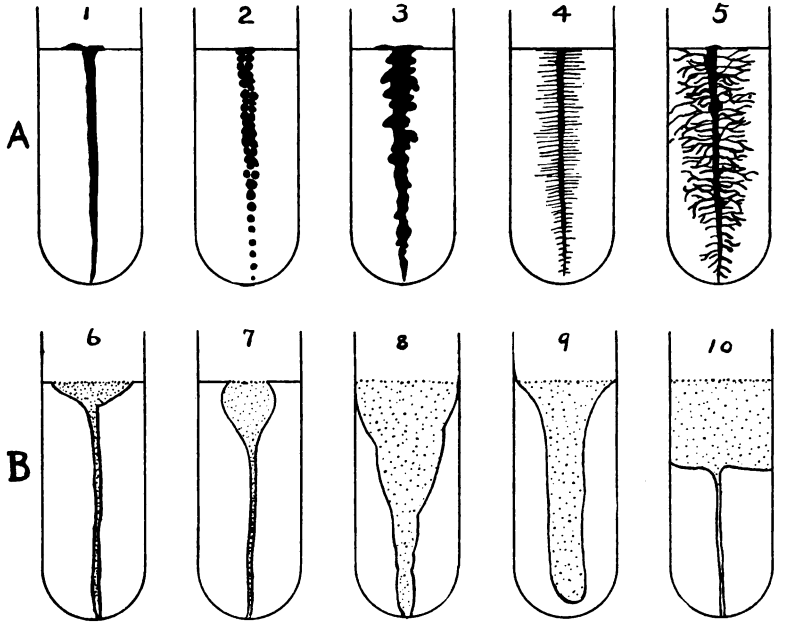


FIG. 18. Types of growth in stab cultures. A. Non-liquefying: 1. Filiform (B. coli) 2. Beaded (Str. pyogenes); 3. Echinate (Bact. acidi-lactici); 4. Villous (Bact. murisepticum); 5. Arborescent (B. mycoides). B. Liquefying: 6. Crateriform (B. vulgare, 24 hours); 7. Napiform (B. subtilis, 48 hours); 8. Infundibuliform (B. prodigiosus); 9. Saccate (Msp. Finkleri); 10. Stratiform (Ps. fluorescens).

II. *Liquefying.*

1. Shape of liquefied area: *crateriform*, saucer shaped (Fig. 18, 6); *napiform*, outline of a turnip (Fig. 18, 7); *infundibuliform*, shape of a funnel, conical (Fig. 18, 8); *saccate*, shape of an elongated sac (Fig. 18, 9); *stratiform*, liquefaction extending to the walls of the tube and then downward horizontally (Fig. 18, 10).

2. Condition of fluid: See Bouillon below.

STREAK CULTURES (Gelatin, Agar, Potato or Blood serum).

1. Form: *filiform* (Fig. 19, 1); *nodose; beaded* (Fig. 19, 3); *papillate*; *echinulate* (Fig. 19, 2); *effused* (Fig. 19, 4); *villous; plumose; arborescent* (Fig. 19, 5).

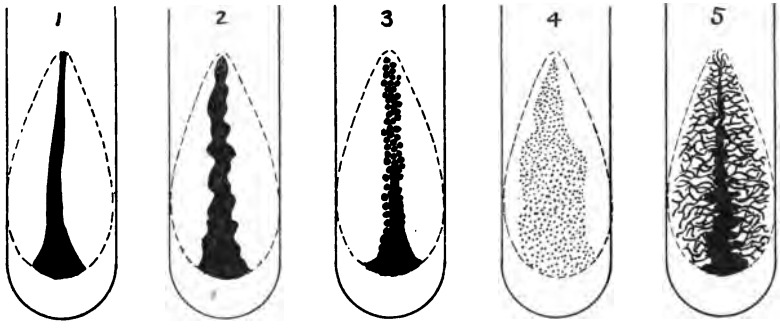


FIG. 19. Types of streak cultures. 1, Filiform (B. coli); 2, Echinulate (Bact. acidilactici); 3, Beaded (Str. pyogenes); 4, Effuse (B. vulgaris); 5, Arborescent (B. mycoides).

2. Size; in millemeters.
3. Surface elevation.
4. Topography of surface.
5. Color.
6. Consistency.
7. Changes in medium.

} Same as plate cultures.

BOUILLON CULTURES.

1. Condition of fluid: *clear; clouded*, degree of, does or does not clear on standing.

2. Membrane: *when formed; color, consistency, structure.*

3. Sediment: *amount; color, character*, whether *compact* or *flocculent*, on agitation appears *granular, flaky* or *viscid.*

4. Reaction.

MILK CULTURES.

I. *Curd formed:*

1. Time required to curdle.

2. Character of curd: *hard* or *soft*; *massed or in fragments; changed or not on boiling.*

3. Whey: *amount; transparent or turbid.*

4. Reaction: *effect on litmus.*

5. Digestion: *time required; solution complete or incomplete; reaction; character of solution*, clear, or cloudy.

6. Gas bubbles.

7. Odor.

II. *Digestion without formation of curd.*

III. *No visible change* even after boiling.

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 or nitrogen.

d. Relation to light, Buchner's Experiment (**46**).

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 in dextrose, lactose, and saccharose media.

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

i. Relation of growth to acidity and alkalinity of medinm: 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 in sugar-free bouillon.

m. Enzym 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. Agglutinating properties of serum of immune animals. (Widal reaction.)
9. Lysogenic properties of serum of immune animals. (Pfeiffer's phenomenon.)

REFERENCES: Chester, Reports Delaware Experiment Station, 1897, 1898 and 1899; A. 216; C. 17; P. B. C. (Cheesman's Charts.)

MIGULA'S SYSTEM OF CLASSIFICATION.

I. Cells globose in a free state, not elongated in any direction before division into 1, 2, or 3 planes. COCCACEAE ZOPF. 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 or 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. Cell 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). Cell division always only in one plane, - - 13. *Streptothrix* COHN.

2). Cell division in three planes previous to the formation of conidia.

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 TREVISAN.

Only one genus. - - 18. *Beggiatoa* TREVISAN.

BACTERIA ARRANGED IN CLASSES AND GROUPS.

Saprophilic Class:
- Bacillus vulgatus Trevisan.
- Bacillus subtilis (Ehrenb.) Cohn.

Chromogenic Class:
- Bacillus prodigiosus (Ehrenb.) Fluegge.

Zymogenic Class:
- Bacillus acidi-lactici Hueppe.

Saprogenic Class:
- Bacillus vulgaris (Hauser) Mig.
- Bacillus Zopfii (Kurth) Mig.

Phosphorescent Class:
- Bacterium phosphorescens (Cohn) Fischer.

Pathogenic Ærobes.

Erysipelas Group:
- Streptococcus erysipelatos Fehleisen.

Pus Coccus Group:
- Micrococcus pyogenes var. albus (Rosenbach) L. & N.
- Micrococcus pyogenes var. aureus (Rosenbach) L. & N.

Malta Fever Group:
- Micrococcus melitensis Bruce.

Diplococcus Group:
- Micrococcus gonorrhœæ (Baum) Fluegge.
- Micrococcus Weichselbaumii (Trevisan.)

Sarcina Group:
- Sarcina tetragena (Gaffky) Mig.

Anthrax Group:
- Bacterium anthracis (Koch) Mig.

Friedlander Group:
- Bacterium pneumonicum (Fried.) Mig.
- Bacterium aerogenes (Esch.) Mig.
- Bacterium capsulatum (Sternberg) Chester.

Swine Plague Group:
- Bacterium choleræ (Zopf) Kitt.
- Bacterium bovisepticum (Kruse) Mig.

Glanders Group:
- Bacterium mallei (Loeffler) Mig.
- Bacterium rhusiopathiæ (Kitt) Mig.

Diphtheria Group:
- Bacterium diphtheriæ (Loeffler) Mig.

Bacterium pseudodiphtheriticum (Loeffler) Mig.

Pneumonia Group:

Bacterium pneumoniæ (Weichsel.) Mig.

Influenza Group:

Bacterium influenzæ (Pfeiffer) Lehm. and Neum.

Tubercle Group:

Bacterium tuberculosis (Koch) Mig.

Bacterium tuberculosis var. avium (Kruse) Mig.

Colon Group:

Bacillus coli (Escherich) Mig.

Bacillus enteritidis Gaertner.

Hog Cholera Group:

Bacillus Salmonii (Trevisan) Chester.

Bacillus icteroides Sanarelli.

Typhoid Group:

Bacillus typhosus Zopf.

Bacillus Shigæ Chester.

Bacillus pestis Lehmann and Neumann.

Pseudomonas Group:

Pseudomonas æruginosa (Shroeter) Mig.

Cholera Group:

Microspira comma (Koch) Schroeter.

Microspira Metschnikovi (Gamaleia) Mig.

Microspira Schuylkilliensis (Abbott) Chester.

Streptothrix Group:

Streptothrix bovis (Harz) Chester.

Streptothrix Maduræ Vincent.

Pathogenic Anærobes.

Emphysema Group:

Bacterium Welchii Mig.

Edema Group:

Bacillus Feseri (Trevisan) Chester.

Bacillus edematis Liborius.

Bacillus botulinus v. Ermengem.

Tetanus Group:

Bacillus tetani Nicolaier.

CHAPTER IV.

SYSTEMATIC STUDY OF REPRESENTATIVE NON-PATHOGENIC BACTERIA.

In making a systematic study of a bacterium it is necessary to determine as many as possible of the points indicated in the previous chapter (III). And in the laboratory this becomes a regular routine procedure—in the study of each germ. The organism is first inoculated into a number of the standard media. These cultures are frequently spoken of as a "set of cultures" and are usually composed of the following: Gelatin and agar plates, a gelatin stab, agar and potato streaks, a bouillon culture (or Dunham's sol.) a milk culture and a dextrose gelatin or agar stab (or shake culture). These cultures are then incubated at the proper temperture for 24 hours. They are then examined, described and sketched. At the same time three cover-glass preparations are made, one each from the agar, bouillon and gelatin cultures and stained with the following dyes: agar with an aqueous solution, bouillon with Loeffler's methylene blue and the gelatin by Gram's method. The bouillon culture is also examined in a hanging-drop for motility and the milk culture for capsules. From these microscopical preparations the morphological characters can usually be determined. The cultures are again placed in the incubator and 24 hours later (48 hours after inoculation) are again examined and any changes are noted and sketched. The cultures are now usually kept at the temperature of the room for about one week and then examined for the last time.

If the organism produces gas in dextrose media, fermentation tubes should be inoculated and the rate, amount and formula of the gas determined.

The descriptions and sketches are conveniently made on the charts provided on the following pages.

EXERCISE 58. 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.
10 tubes of dextrose agar or gelatin.
20 tubes of gelatin.
10 tubes of bouillon.
10 fermentation tubes of dextrose bouillon.
10 tubes of potato.
10 tubes of milk.
10 tubes of sugar-free bouillon, or Dunham's solution.
10 water-blanks.

Bacillus vulgatus Trevisan.

SYNONYMS. Bacillus mesentericus vulgatus Fluegge.

COMMON NAME. Potato bacillus.

EXPLANATORY. This is a widely distributed organism which was first described by Fluegge in 1886. Its spores are very resistant and can almost invariably be found on potatoes. It can usually be obtained by boiling potatoes for a half an hour, halving them and incubating in a sterile moist chamber.

REFERENCES. Fluegge: Die Mikroorganismen, 1886; C. 271; L. & N. 323; Mig. 2: 556.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

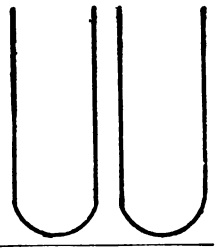
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

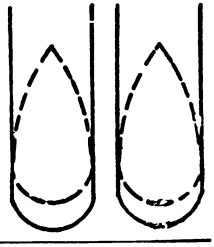
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

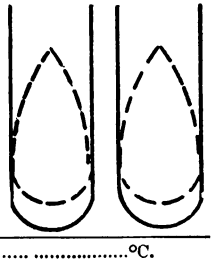
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

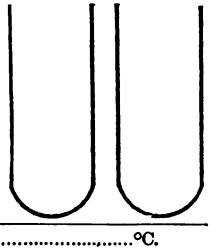
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2$: : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus subtilis (Ehrenb.) Cohn.

SYNONYMS. Vibrio subtilis Ehrenberg.

COMMON NAME. Hay bacillus.

EXPLANATORY. This is a well-known and widely distributed organism. First described by Cohn in 1872. It is almost invariably found on hay, hence the common name. Its spores, like the "potato bacillus," are very resistant to heat. A pure culture can usually be obtained by making an infusion of hay or straw and heating it to 80° C. for ten minutes.

REFFRENCES. F. Cohn, Beitraege Zur Biologie, Bd. I, 1872, Heft 2, p. 175; C. 276; L. 170; L. & N. 317; Mig. 2: 515.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

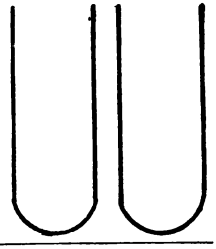
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

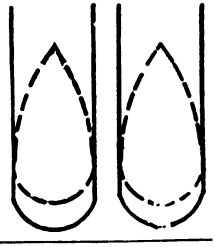
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

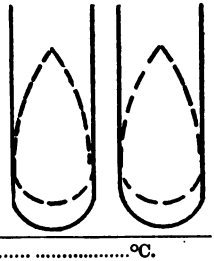
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

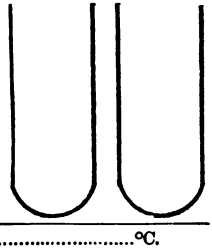
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus prodigiosus (Ehrenb.) Fluegge.

SYNONYMS. Monas prodigiosa Ehrenb.; M. prodigiosus Cohn.

EXPLANATORY. This organism was first described by Ehrenberg. It is the oldest known chromogenic bacterium. It is very 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 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 edema 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. Ehrenberg, Erhandlunger der Berliner Akademie, 1839; C. 258; L. 137; L. & N. 272; Mig. 2: 845.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

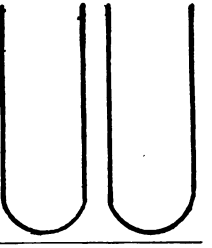
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

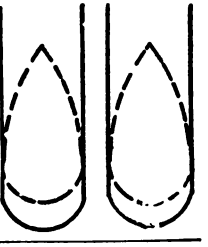
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

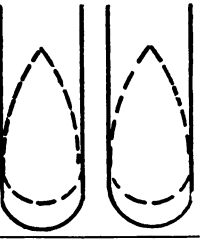
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

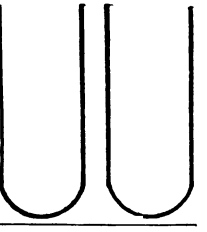
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

EXERCISE 61. VARIETY OF PIGMENTS.

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

	3 TO 6 DAYS.	SKETCHES.
Bacillus indicus or		
Sarcina aurantiaca or		
Sarcina lutea or		
Pseudomonas fluorescens (B. fluorescens) or		
Pseudomonas aeruginosa (B. pyocyaneus or		
Pseudomonas violacea or		

EXERCISE 62. 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.

Bacterium acidi-lactici Zopf.

COMMON NAME. Lactic acid bacillus.

EXPLANTTORY. This organism may be taken as a type of the bacteria causing sour milk of which there are a very large number. It was first described by Hueppe in 1884. It is very widely distributed.

REFERENCES. Hueppe, Mitteil, aus dem Kaiserl. Gesundheitsamte, 1884, Bd. II. p. 1837; C. 149; Cn. 189; L. 222; L. & N. 220. Mig. 2: 327.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon.....	
.....	
.....	
b. Agar.....	
.....	
.....	
c. Gelatin	
.....	
.....	
d. Other media	
.....	
2. Size:.....	
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:	
a. Capsules.....	
b. Involution forms.....	
c. Deposits or vacuoles.....	
d. Pleomorphism.....	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

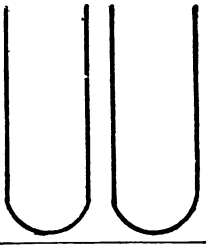
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

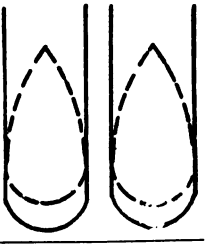
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

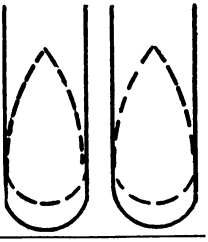
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

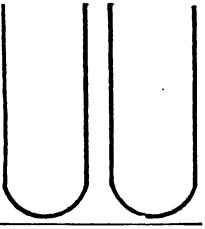
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT .....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus vulgaris (Hauser) Mig.

SYNONYMS. Proteus vulgaris Hauser. B. proteus Trevisan.

EXPLANATORY. First described by Hauser. 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. Hauser, Ueber Faulnisbakterien, 1885; C. 244; Lafar 194–199. Mig. 2: 707.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

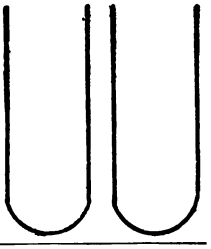
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

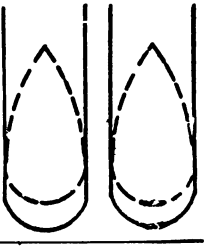
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

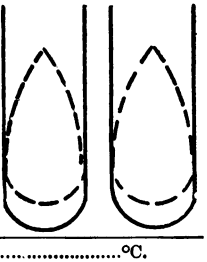
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

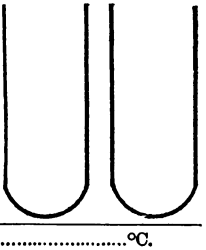
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, H : CO_2 : : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus Zopfii (Kurth) Mig.

SYNONYMS. Bacterium Zopfii Kurth; Proteus Zenkeri Hauser.

EXPLANATORY. This organism belongs to the group of putrefactive bacteria (proteus group.)

REFERENCES. Kurth, Botan. Zeitung., 1883; C. 248; Mig. 2: 815.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

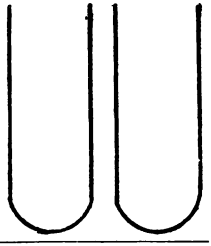
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

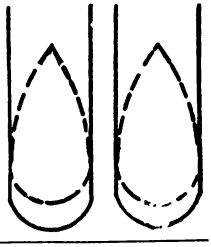
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

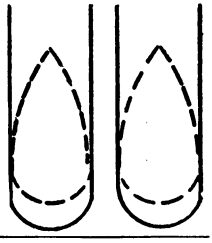
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

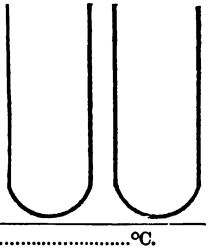
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1 Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium phosphorescens (Cohn) Fischer.

SYNONYMS. Photobacterium phosphorescens Beijernck.

EXPLANATORY. Fisst described by Fischer in 1887. Found in Kiel harbor on 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. Fisher, Zeitschrift fur Hygiene, 1887, Band 2, p. 92; C. 181; L. & N. 231; Mig. 2: 433.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

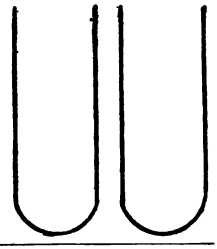
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

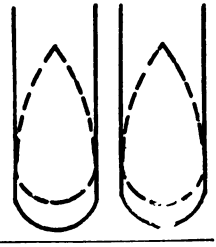
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

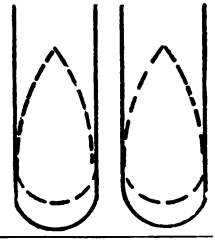
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

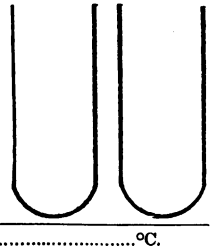
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT .....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

CHAPTER V.

BACTERIOLOGICAL ANALYSIS.

EXERCISE 66. COMPARATIVE ANALYSIS OF AIR (KOCH).

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. The counting is facilitated by the use of Plate II. on which the Petri dishes are to be placed. In counting a hand lens magnifying about 5 diameters should be used. Where possible all of the colonies on the plate should be counted, if this be impossible count a representative area and estimate the whole number.

d. Express the results in terms of the number of organisms which fall per square foot per minute. The area of the Petri dish can be read off directly from Plate II. in square centimeters, or it can be calculated by multiplying the square of the diameter by O. 785.

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.

REFERENCE. H. 390.

EXERCISE 67. QUANTITATIVE DETERMINATION OF NUMBER OF BACTERIA IN AIR (PETRI-SEDGWICK).

GENERAL DIRECTIONS.

a. A piece of glass tubing 6 mm. ($\frac{3}{8}$ 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. 22, A).

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

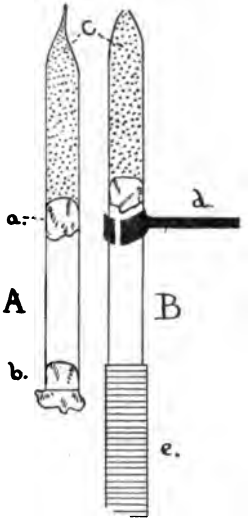


FIG. 22. Apparatus for filtering air through sugar. A, ready for sterilization. B, point broken off and attached to aspirator.

d. Fasten the tube, pointed end up, in a clamp, remove the first cotton plug and connect with an aspirator (Fig. 23).

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

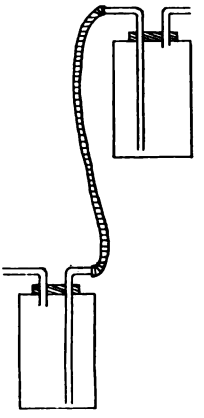


FIG. 23. Aspirator for filtering air.

SPECIAL DIRECTIONS.

a. Filter 50 liters of air.

b. Dissolve sugar in 10 cc. of sterile water (water-blank) 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. 228; N. 449 S. 541.

EXERCISE 68. WATER ANALYSIS.

COLLECTION. Water for analysis must be collected in a sterile vessel. A test-tube or flask may be used in the laboratory, but when the collection is made outside a sterile glass-stoppered bottle should be used. In collecting, special care should be taken to get a fair sample; if the water be in a reservoir, or the like, the bottle should be filled below the surface to avoid the scum and away from the bottom to avoid the sediment. Fig. 24 shows a form of apparatus used to take samples in deep water. If some time must necessarily elapse between the collection of the sample and its examination it should be packed in ice. Specially constructed shipping cases are used in most laboratories.

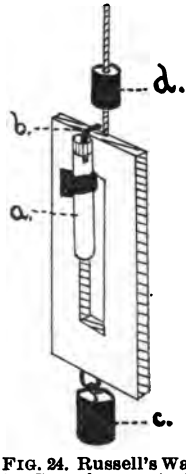


FIG. 24. Russell's Water Sampler. *a*, test-tube from which the air has been exhausted; *b*, glass tube, sealed; *c*, sinker; *d*, weight to be dropped at proper depth.

QUANTITATIVE ANALYSIS.

a. After shaking the sample at least 25 times remove 1 cc. of the water by means of a sterile pipette and place it in the bottom of a sterile Petri dish. In the same way remove ½ cc. and $\frac{1}{10}$ cc. Pipettes graduated to $\frac{1}{10}$ths. may be used, or a 1 cc., or even a 5 cc. pipette may be used by counting the whole number of drops delivered and then taking the required fraction. If the sample be supposed to be highly infected it should be diluted with sterile water before the cultures are made. Plates ought not to contain over 200 colonies.

b. Pour into the dishes fluid gelatin (not warmer than 42° C.) and tip them from side to side until the medium and water are thoroughly mixed. Solidify and incubate at 22° C., or below.

c. In the same way make agar plates using ordinary agar or, better, 5% glycerine agar. Incubate at 22° C.

d. Count the colonies at the end of 48 hours as directed above (**66** *c.*) and at intervals afterward until the maximum number of colonies is obtained. Express the results in the number of bacteria per cc. of water.

QUALITATIVE ANALYSIS.

a. Number of species. Examine carefully, under the low power of the microscope, the plates made above to determine the number of different species, describing each very briefly. Estimate also the total number of liquefying organisms per cc.

b. Search especially for proteus forms, i. e. colonies of *B. vulgaris* (**64**).

c. Tests for Fecal Bacteria (*B. coli*).

1. Fermentation tube test. Inoculate several fermentation tubes with from 1 to 2 cc. of water and incubate at 38° C. Tubes which develop from 30 to 70% of gas should have plate cultures made from them and then the gas formula may be determined. For *B. coli* it will be about $\frac{H}{CO_2} = \frac{2}{1}$.

2. Indol test. Tubes of sugar-free bouillon or of Dunham's solution inoculated and incubated at 38° C. for 48 to 96 hours will show the presence of indol if *B. coli* be present.

3. Acid colonies. A lactose-litmus agar plate should be made (using about 1 cc. of water) and kept at 38° C. Examine 24 hours later for acid colonies.

d. Pathogenic Bacteria. See Chapter X.

REFERENCES. A. 526; H. 373; L. & K. 396; McF. 233; M. & R. 79; N. 422; P. 245; S. 553. For the determination of the vari-

ous species present see Frankland's Micro-organisms of Water; Fuller: Report Am. Public Health Assoc., 1899, 580; Chester.

SPECIAL DIRECTIONS. Analyze a surface water (lake or river), a deep well or a spring water.

EXERCISE 69. 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. 25.)

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. 238; N. 444; S. 567.

FIG. 25. Fraenkel's Soil Borer.

EXERCISE 70. 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 (**69**).

d. Count colonies and estimate number of bacteria per cc.

EXERCISE 71. 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 (Fig. 26) 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.

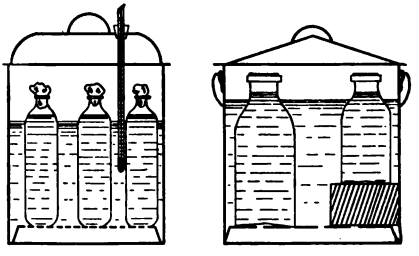


FIG. 26. Home-made pasteurizers.

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. Wis. Exp. Station Bull. No. 44 and 18th. An. Rept. 185. Russell, Outlines of Dairy Bacteriology, (5th Edit.) 113.

EXERCISE 72. 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 be not volatile, or affected by heat, sterilize.

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

d. Keep these cultures under observation in the 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 73. 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* or *M. pyogenes.*

COVER-GLASS METHOD.

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

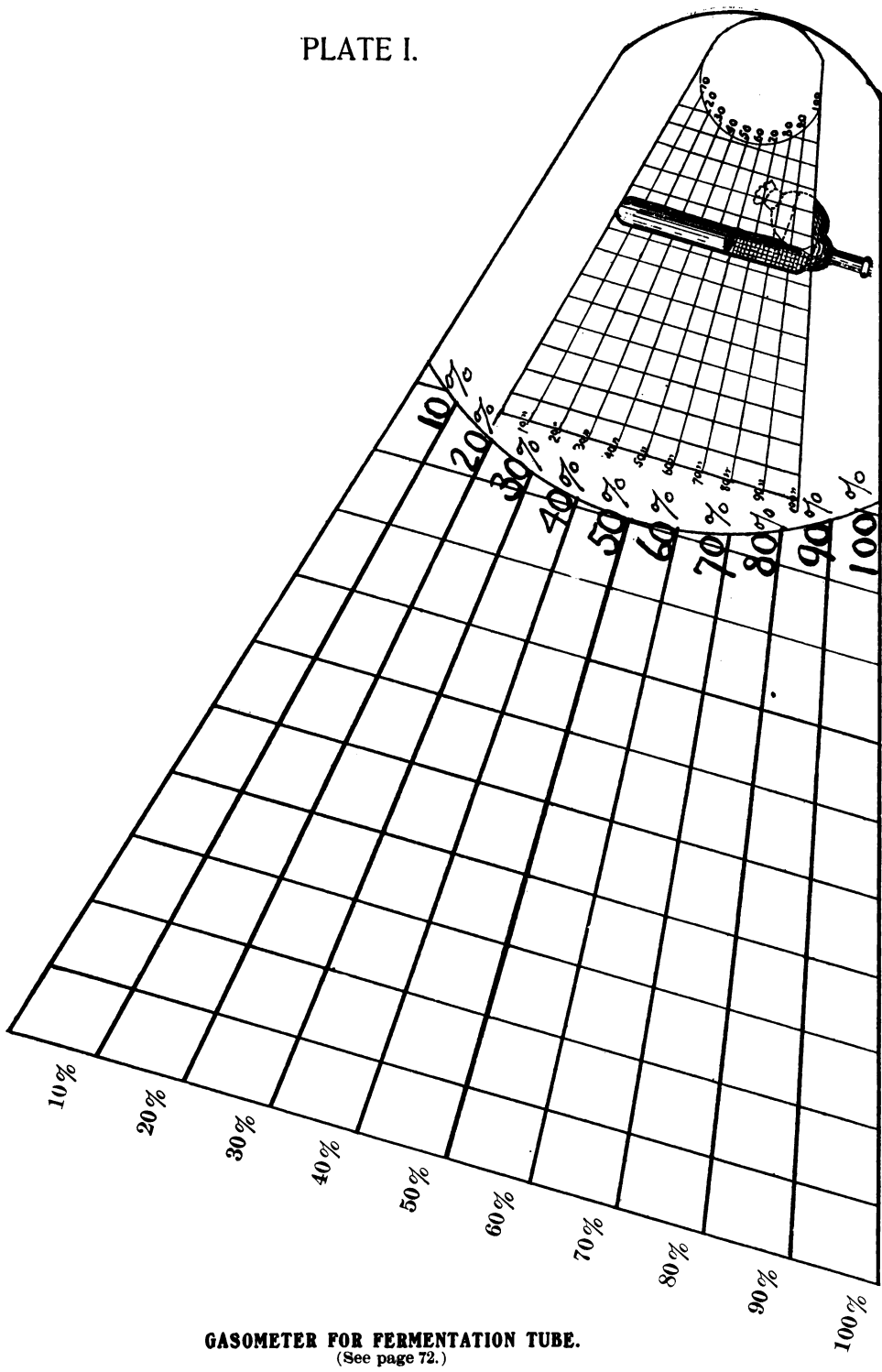
b. By means of a burette, pipette, or loop, place the same sized drop on each of several sterile cover-glasses and dry as directed in the experiment on dessiccation (**43**).

c. When the cover-glasses are dry, they are to be immersed in the disinfectant for the stated periods of time; then removed, washed in sterile water 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.

PLATE I.



GASOMETER FOR FERMENTATION TUBE.

(See page 72.)

PLATE II.

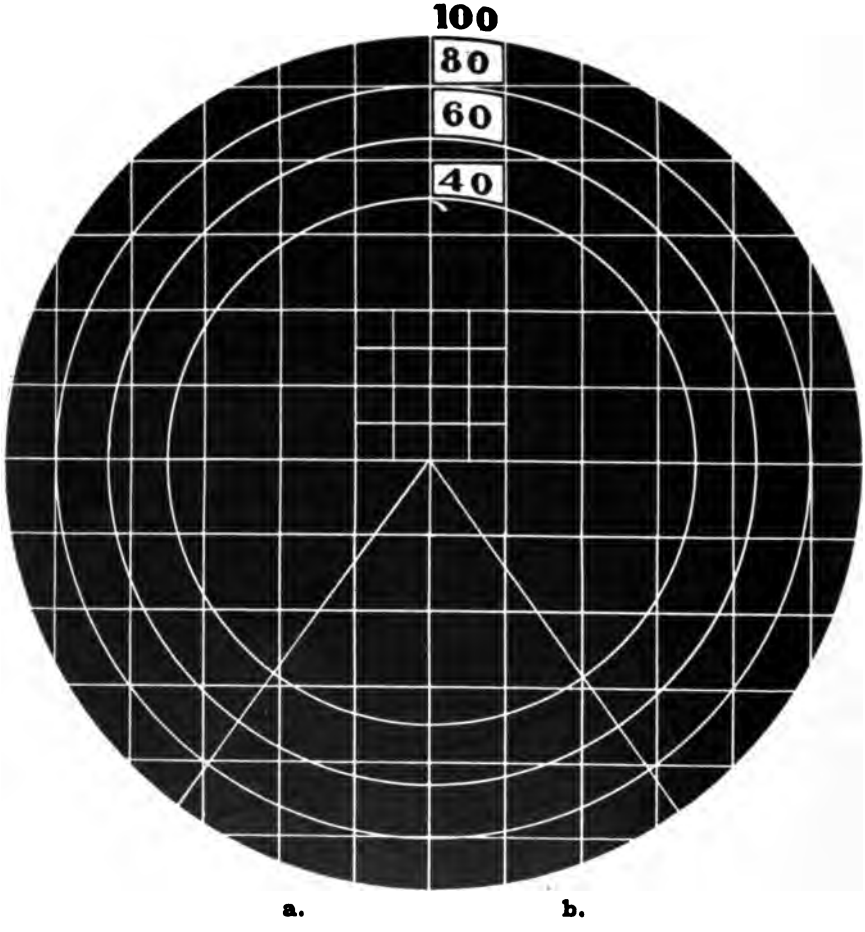


PLATE COUNTER (Modified from Jeffers).
FOR COUNTING COLONIES OF BACTERIA.

The cross lines divide the figure into square centimeters. The numbers indicate the area of the various discs. The area of each sector (a. and b.) is one-tenth of the whole area. (See page 122.)

PART II.

MEDICAL BACTERIOLOGY.

PART II.—MEDICAL BACTERIOLOGY.

CHAPTER VI.

PATHOGENIC AEROBES.

EXERCISE 74. 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 dextrose agar.
100 tubes of gelatin.
12 tubes of dextrose gelatin.
30 tubes of bouillon.
10 fermentation tubes of dextrose bouillon.
35 tubes of potato.
35 tubes of milk.
35 tubes of dextrose free broth or Dunham's solution.
30 water blanks.
30 tubes of blood serum. This is prepared as follows.

a. Collection of the blood. Sterilize Mason fruit jars, by successive washings in corrosive sublimate, distilled water, alcohol and ether (or a large pail may be used). These are to be carried to the slaughter house and the blood from a beef caught directly into them. The blood is then allowed to stand undisturbed for 15 to 30 minutes, or until the clot has firmly attached itself to the sides of the vessels, when they are to be covered and 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 ½% 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 1% dextrose bouillon.

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 heating 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 is 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 be employed bubbles are formed which rupture the surface of the medium in their escape. When steriled the tubes should be sealed with paraffin or otherwise.

REFERENCES. A. 106; H. 45; L. &. K. 83; M. & R. 50; M. & W. 81; McF. 94; N. 463; P. 219; S. 37 & 55.

Streptococcus erysipelatos Fehleisen.

SYNONYM. Streptoccus pyogenes Rosenbach.

COMMON NAMES. Streptococcus; chain coccus.

EXPLANATORY. First described by Fehleisen. 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. Fehleisen, Aetiol. des Erysipels, Berlin 1883; A. 268; C. 65; Fl. 2, 106; H. 133; L. & K. 117; L. & N. 135; Mig. 2, 6; M. & R. 168; M. & W. 124; McF. 254; P. 476; S. 274.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

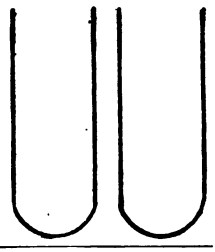
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

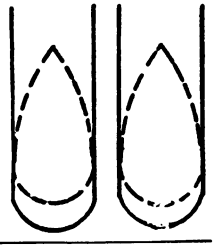
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

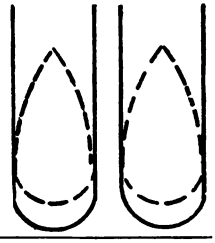
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

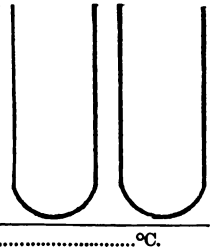
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2$: : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Micrococcus pyogenes var. **albus** (Rosenbach) L. & N.

SYNONYMS. Staphylococcus pyogenes albus Rosenbach; Staphylococcus epidermidis albus Welch.

COMMON NAMES. Grape coccus; pus coccus; white staphylococcus.

EXPLANATORY. First described by Rosenbach. 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; C. 75; Fl. 2, 105; L. & N. 180 Mig. 2, 87; McF. 254; P. 470; S. 272.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

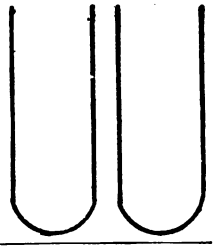
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

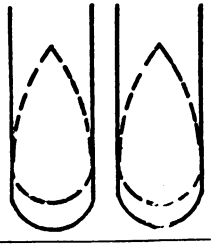
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

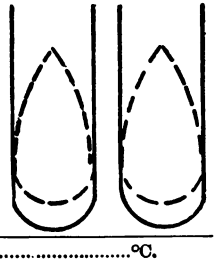
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

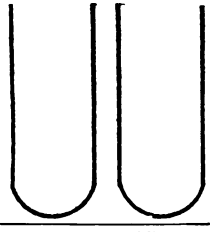
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1 Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Micrococcus pyogenes var. **aureus** (Rosenbach) L. & N.

SYNONYM. Staphylococcus pyogenes aureus (Rosenbach).

COMMON NAME. Golden pus coccus.

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

REFERENCES. Rosenbach, Mikroorganismen bei dem Wundinfektionskrankheiten des Menschen, 1884; A. 260; C. 89; Fl. 2, 96; H. 130; L. & K. 115; L. & N. 180; Mig. 2, 135; M. & R. 166; M. & W. 121; McF. 249; P. 461; S. 265.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

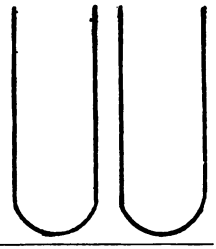
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

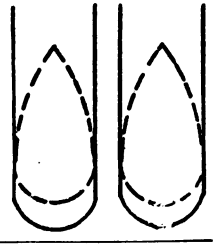
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

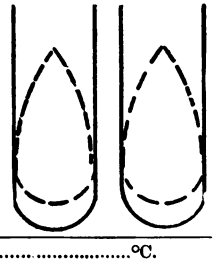
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

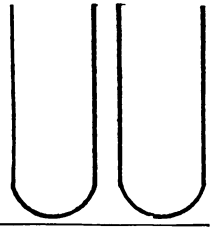
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Micrococcus melitensis Bruce.

COMMON NAME. Coccus of Malta fever.

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

REFERENCES. Bruce, Practitioner, Sept. 1887 and Ann. de l' Inst. Pasteur, 1893, 7, 289; Durham, Jour. Path. and Bact., 1898, 5, 377; H. 361; L. & N. 168; Mig. 2, 83; M. & R. 449.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

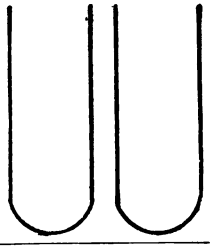
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

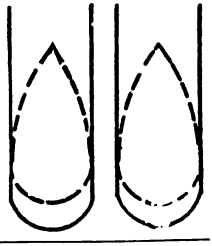
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

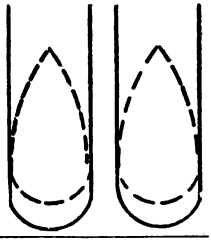
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

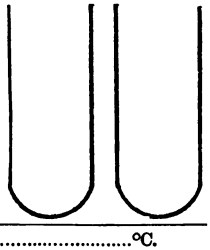
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Micrococcus gonorrhoeae (Baum) Fluegge.

SYNONYM. Diplococcus gonorrhœæ Baum.

COMMON NAME. Gonococcus.

EXPLANATORY. 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 (gonorrhœal rheumatism), endocarditis, salpingitis and general septicemia.

REFERENCES. Neisser, Cent. f. d. Mediz. Wissensch., 1879, 497; Foulerton, Trans. Brit. Inst. of Prev. Med., 1897, 1, 40; A. 277; C. 72; Fl. 2, 149; H. 145; L. & K. 311; L. & N. 164; Mig. 2, 188; M. & R. 189; M. & W. 130; McF. 266; P. 522; S. 283.

CULTURE CHARACTERS.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

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.

Micrococcus Weichselbaumii (Trevisan).

SYNONYM. Diplococcus intracellularis meningitidis Weichselbaum.

EXPLANATORY. 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. Weichelbaum, Fortschritte der Medicine, 1887; Councilman, Rept. Mass. State B. of H. 1898; A. 285; C. 64; Fl. 2, 144; H. 138; L. & N. 148; Mig. 2, 189; M. & R. 172; M. & W. 135; P. 516; S. 310.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

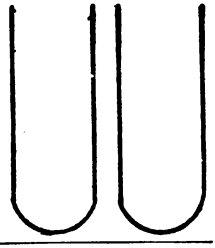
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

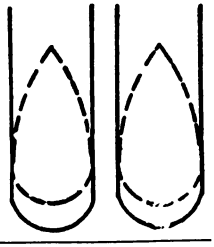
Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

Gelatin Stab: GROWN 24 HOURS AT.....°C.



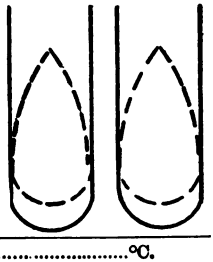
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Agar Streak: GROWN 24 HOURS AT.....°C.



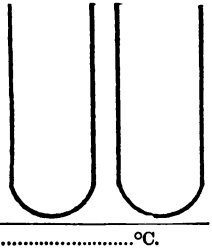
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C. | 6 DAYS AT.....°C.

1 Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Sarcina tetragena (Gaffky) Mig.

SYNONYM. Micrococcus tetragenus Gaffky.

EXPLANATORY. First described in 1883 by Gaffky. It is found in phthisical cavities and in 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; C. 84; Fl. 2, 155; H. 139; L. & N. 171; Mig. 2, 225; M. & R. 171; M. & W. 133; McF. 563; P. 472; S. 314.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

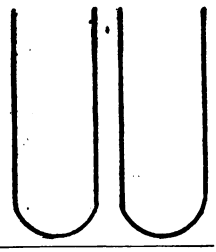
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

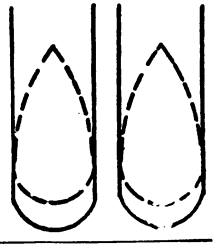
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

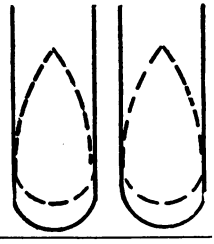
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

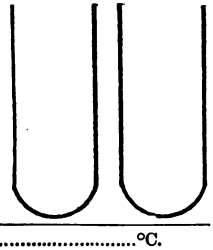
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium anthracis (Koch) Mig.

SYNONYM. Bacillus anthracis Koch.

COMMON NAME. Anthrax bacillus.

EXPLANATORY. 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; C. 190; Fl. 2, 217; H. 151; L. & K. 287; L. & N. 307; Mig. 2, 280; M. & R. 295; M. & W. 156; McF. 455; P. 547; S. 328.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

CULTURE CHARACTERS

Reaction of media: Fuller's scale — ________ or — ________

Gelatin plate: grown 24 hours at ________ °C. SKETCH

a. Surface Colonies

b. Deep Colonies

---

48 hours at ________ °C. 6 days at ________ °C.

Agar plate: grown 24 hours at ________ °C. SKETCH

a. Surface Colonies

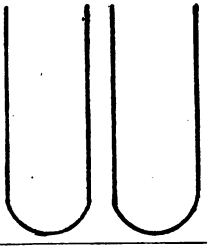
b. Deep Colonies

---

48 hours at ________ °C. 6 days at ________ °C.

Special Media: such as [illegible]

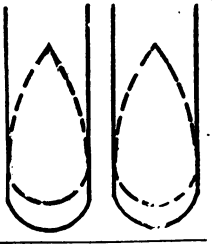
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

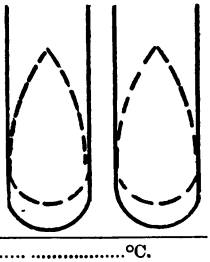
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

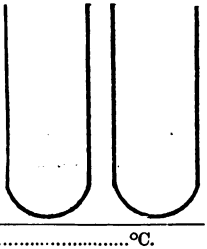
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

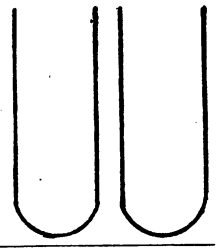
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

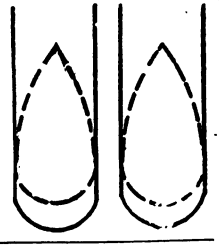
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

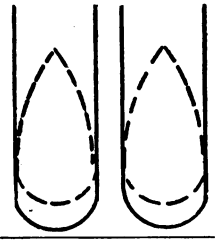
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

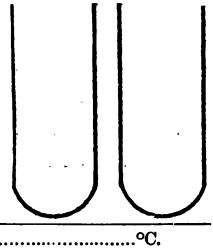
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium pneumonicum (Fried.) Mig.

SYNONYMS. Bacillus pneumoniæ Weichselbaum; Pneumobacillus Friedlander.

COMMON NAME. Friedlander's bacillus.

EXPLANATORY. 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; C. 131; Fl. 2, 342; H. 278; L. & K. 119; L. & N. 225; Mig. 2, 350; M. & R. 211; McF. 287; P. 458; S. 296.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

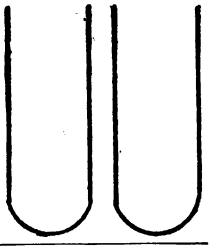
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

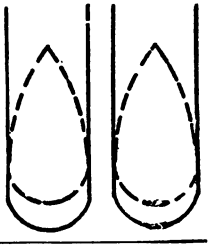
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

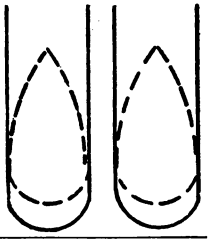
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

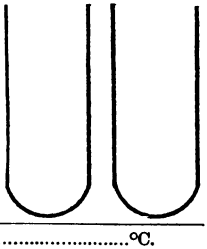
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, H : CO_2 : : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium aerogenes (Esch.) Mig.

SYNONYMS. Bacterium lactis aerogenes Escherich; Bacillus aerogenes Kruse.

EXPLANATORY. This organism was first described by Escherich who isolated it from the milk-stools of infants. It is very similar to Bact. acidi-lactici, and often difficult to differentiate from B. coli. Found in milk, feces, air, water, etc.

REFERENCES. Escherich, Fortschritte der Medizin, 1885, No. 16–17. C. 128; Fl. 2, 340; L. & N. 221; Mig. 2, 396.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

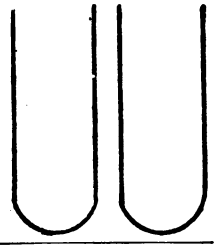
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

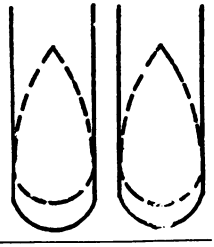
Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

Gelatin Stab: GROWN 24 HOURS AT.....°C.



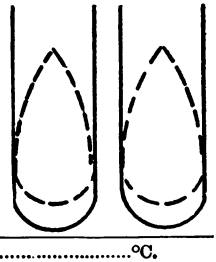
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Agar Streak: GROWN 24 HOURS AT.....°C.



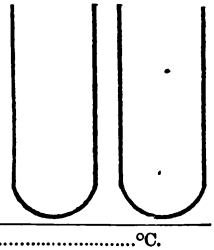
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C. | 6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium capsulatum (Sternberg) Chester.

SYNONYMS. Bacillus capsulatus Sternberg.

COMMON NAME. Pfeiffer's capsule bacillus.

EXPLANATORY. First described by Pfeiffer who isolated it from the blood of guinea pigs which died spontaneously.

REFERENCES. Pfeiffer, Z. f. H. 1889, 6, 145; C. 129; L. & N. 228; Mig. 2, 349.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

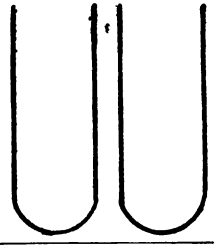
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

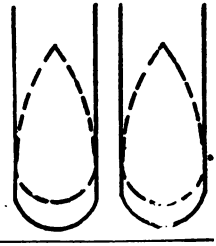
Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

Gelatin Stab: GROWN 24 HOURS AT.....°C.



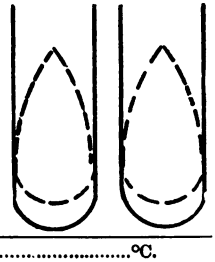
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Agar Streak: GROWN 24 HOURS AT.....°C.



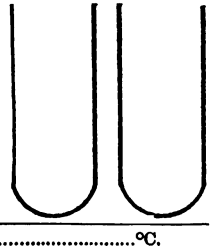
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C. | 6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium cholerae (Zopf) Kitt.

SYNONYMS. Bacterium choleræ-gallinarum Crookshank; Bact. suicida Migula; Bacillus Septicemiæ hemorrhogicæ Hueppe.

COMMON NAMES. Bacillus of chicken cholera; Bacillus of swine plague.

EXPLANATORY. First described by Koch in 1878. Found in blood, organs and excreta of chickens suffering with fowl cholera, and in 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; C. 135; Fl. 2, 413; H. 268; L. & N. 208; Mig. 2, 364; McF. 527; S. 408.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

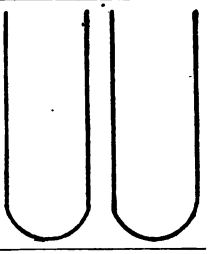
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

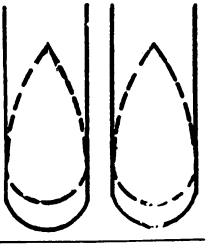
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

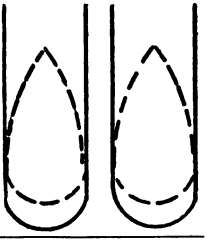
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

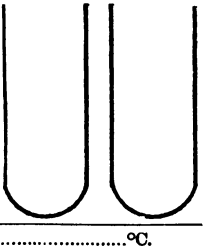
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium bovisepticum (Kruse) Mig.

SYNONYM. Bacillus bovisepticus Kruse.

COMMON NAME. Bacillus of Hemorrhogic septicemia in cattle.

EXPLANATORY. First described by Bollinger, 1878. It is the cause of hemorrhogic septicemia in cattle and in other animals.

REFERENCES. Bollinger, Ueber eine neue Wild und Rinderseuche, Muenchen, 1878; C. 137; Fl. 2, 421; Mig. 2, 367.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

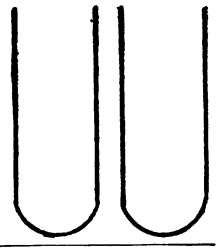
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

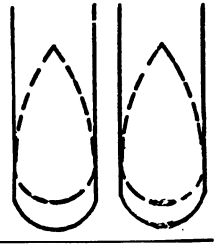
Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

Gelatin Stab: GROWN 24 HOURS AT.....°C.



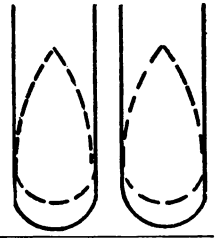
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Agar Streak: GROWN 24 HOURS AT.....°C.



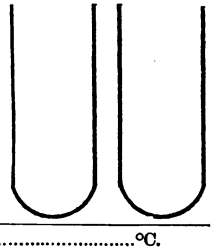
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C. | 6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium mallei (Loeffler) Mig.

SYNONYM. Bacillus mallei Loeffler.

COMMON NAME. Bacillus of Glanders.

EXPLANATORY. 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; L. & N. 384; Mig. 2, 498; M. & R. 268; M. & W. 164; McF. 248; P. 598; S. 396.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C. | SKETCH.

(a) Surface Colonies.

(b) Deep Colonies.

48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C. | SKETCH.

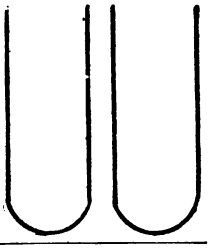
(a) Surface Colonies.

(b) Deep Colonies.

48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

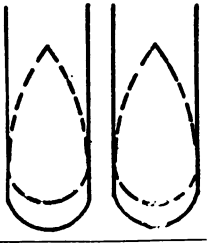
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

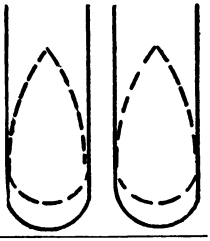
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

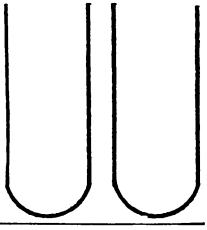
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT .....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium rhusiopathiae (Kitt) Mig.

SYNONYSM. Bacterium erysipelatus-suis Migula.

COMMON NAME. Bacillus of swine erysipelas.

EXPLANATORY. 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; C. 352; Fl. 2, 442; L. & N. 300; Mig. 2, 431; McF. 426; S. 420.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

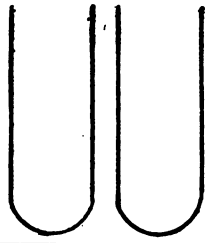
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

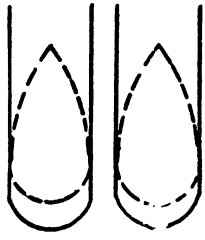
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

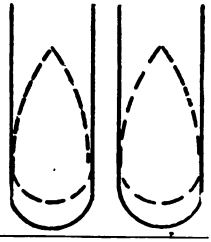
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

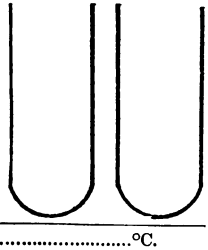
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium diphtheriae (Loeffler) Mig.

SYNONYM. Bacillus diphtheriæ Loeffler.

COMMON NAMES. Diphtheria bacillus; Klebs-Loeffler bacillus.

EXPLANATORY. 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; C. 354; Fl. 2, 460; H. 162; L. & K. 207; L. & N. 389; Mig. 2, 499; M. & R. 353; M. & W. 137; McF. 344; P. 229; S. 356.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) + ... or − .....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

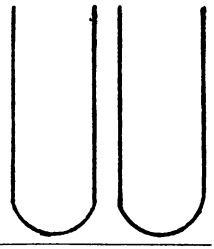
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

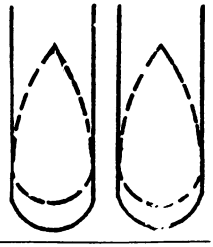
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

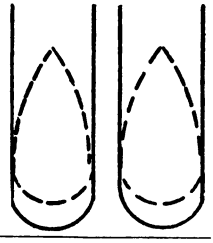
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

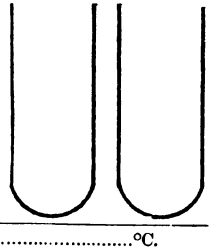
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium pseudodiphtheriticum (Loeffler) Mig.

SYNONYMS. Pseudodiphtheria bacillus of Loeffler; xerose bacillus of Neisser–Kuschbert.

EXPLANATORY. Isolated by Hoffman and others, from the healthy mouth and throat; by Neisser–Kuschbert and others from xerosis and other affections of the conjunctiva. This may be only a non-virulent variety of Bact. diphtheriae.

REFFRENCES. C. 355; Fl. 2, 476; L. & N. 404; Mig. 2, 503.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... **or** −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

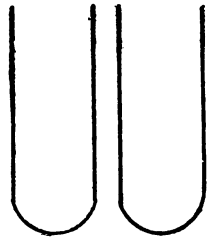
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

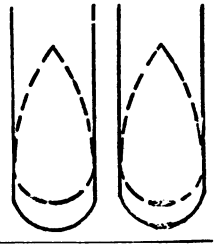
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

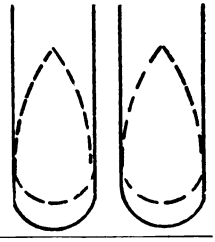
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

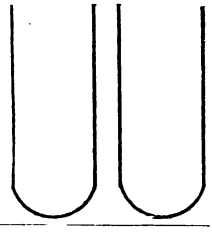
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacterium pneumoniae (Weichselbaum) Mig.

SYNONYMS. Diplococcus pneumoniæ Weichselbaum; Streptococcus lanceolatus Gamaleia.

COMMON NAMES. Pneumococcus; Fraenkel's pneumonia coccus.

EXPLANATORY. First described by Sternberg in 1880. Found in saliva and in the nasal secretion of healthy persons—from 20 to 50 per cent. Usually present in "rusty sputum" of pneumonia patients.

REFERENCES. Weichselbaum, Wiener Med. Jahrbuecher, 1886; Welch, Johns Hop. Hosp. Bulletin, 1892, 3, 125; A. 303; C. 63; Fl. 2, 115; H. 273; L. & K. 118; L. & N. 143; Mig. 2, 347; M. & R. 204; M. & W. 128; McF. 279; P. 498; S. 298.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

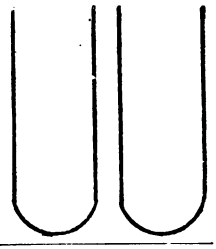
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

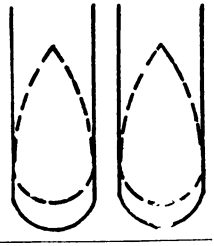
Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

Gelatin Stab: GROWN 24 HOURS AT.....°C.



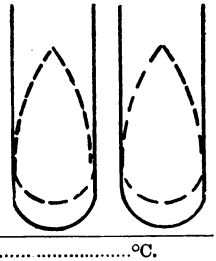
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Agar Streak: GROWN 24 HOURS AT.....°C.



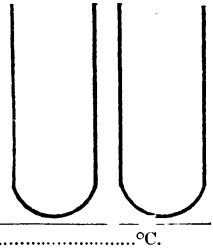
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT .....°C. | 6 DAYS AT.....°C.

1. Relation to temperature: .....
   optimum.....°C.; limits..... to.....°C.;
   thermal death-point.....°C.; time of exposure.....minutes.;
   medium in which exposure is made.....
2. Relation to free oxygen: .....
.....
3. Relation to other agents, such as.....
   desiccation, light, disinfectants, etc: .....
.....
.....
4. Pigment production: .....
.....
5. Gas production in sugar media: .....
   a. dextrose (1) Shake culture.....
   (2) Fermentation tube, growth in open arm.....closed arm.....
   rate of development: 24 hours.....per cent., 48 hours..... per cent.
   72 hours.....per cent., ...hours.....per cent.
   reaction in open arm.....
   gas formula, $H : CO_2$: : ..... : .....
   b. lactose..... *c.* saccharose.....
6. Acid or alkali production: .....
.....
   litmus milk .....
.....
7. Reduction of nitrates: .....
   to nitrites..... to ammonia.....
8. Indol production: .....
   48 hours.....days .....
9. Enzyme production: .....
.....
   proteolytic.....
   digestion of gelatin..... digestion of casein.....
   diastatic.....
.....
10. Characteristic odor: .....
11. Pathogenesis (or other special characters): .....
.....
.....
.....
.....
.....
.....

Bacterium Influenzae (Pfeiffer) L. & N.

SYNONYM. Bacillus influenzæ Pfeiffer.

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

REFERENCES. Pfeiffer, Z. f. H. 1993, 13; 357; A. 334; C. 351; Fl. 2; 434; H. 280; L. & K. 281; L. & N. 202; Mig. 2, 506; M. & R. 431; M. & W. 162; McF. 566; P. 320; S. 370.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

B. *influenzae* does not grow on the ordinary artificial culture media, but may be cultivated on agar slopes upon the surfaces 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.

Bacterium tuberculosis (Koch) Mig.

SYNONYM. Bacillus tuberculosis Koch.

COMMON NAME. Tubercle bacillus.

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

REFERENCES. Koch, Berlin. Klin. Wochenschr., 1882, 15, 221; Smith: Jour. Exp. Med., 1898, 3, 451; A. 312; C. 356; Fl. 2, 481; H. 189; L. & K. 251; L. & N. 410; Mig. 2, 492; M. & R. 324; M. & W. 148; McF. 292; P. 623; S. 375.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Bact. tuberculosis does not grow upon the ordinary artificial media, but may be grown upon blood serum [see p. 134 (1)] and bouillon, agar and potato to which 5% of glycerine has been added. The tubercule 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 dessication, 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.

Bacterium tuberculosis var. **avium** (Kruse) Mig.

SYNONYM. Bacillus tuberculosis avium Kruse.

COMMON NAME. Bacillus of fowl tuberculosis.

EXPLANATORY. This organism was first separated from T. B. by Maffucci, and is probably only a variety of the latter. It is pathogenic for fowl, but ordinarily not for other animals.

REFERENCES. Maffucci, Z. fur H., 1892, 11, 445; C. 356; Fl. 2, 506; L. & N. 418; Mig. 2, 495.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Culture requirements practically the same as for Bact. tuberculosis.

Bacillus coli (Escherich) Mig.

SYNONYM. Bacterium coli commune Esch.

COMMON NAME. Colon bacillus; coli Escherich.

EXPLANATORY. Escherich was the first to describe this organism which is widely known as a common inhabitant of the colon of man, and of some of the lower animals.

REFERENCES. Escherich, Darmbak. des Säuglings, Stuttgart, 1886; C. 205; Fl. 2, 363; L. & N. 243; Mig. 2, 734.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

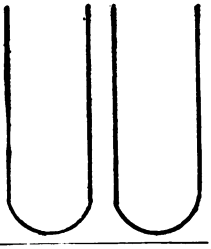
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

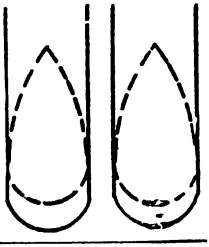
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

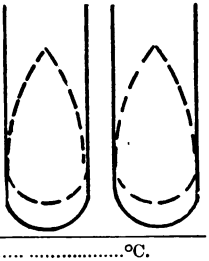
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

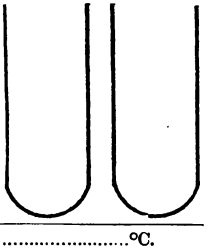
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus enteritidis Gaertner.

EXPLANATORY. Isolated by Gaertner from beef in meat poisoning case, very closely related to preceding.

REFERENCES. Gaertner, Correspond. d. allg. Artze Vereins, Thuringen, 1888; C. 207; Fl. 2, 375; L. & N. 251; Mig. 2, 744.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

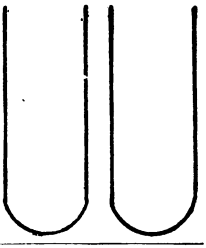
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

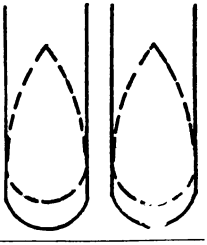
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

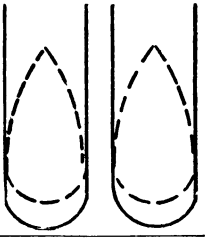
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

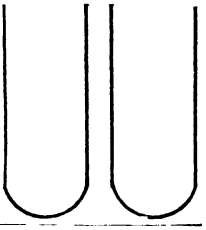
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus Salmonii (Trevisan) Chester.

SYNONYMS. B. suipestifer Kruse; Bact. cholera-suum Lehn. & Neum.

COMMON NAME. Hog-cholera bacillus.

EXPLANATORY. 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 and Smith, Rept. Bureau Anim. Ind., 1885–91; C. 210; Fl. 2, 401; H. 269; L. & N. 252; Mig. 2, 759; McF. 531; S. 413.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) + ... or − .....

Gelatin plate: GROWN 24 HOURS AT.....°C. | SKETCH.

(a) Surface Colonies.

(b) Deep Colonies.

48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C. | SKETCH.

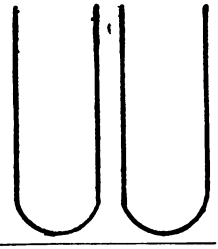
(a) Surface Colonies.

(b) Deep Colonies.

48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

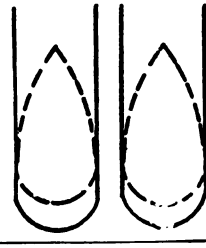
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

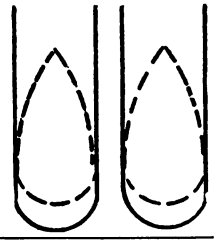
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

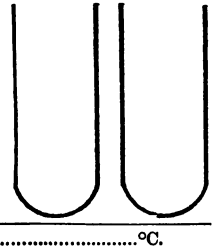
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus icteroides Sanarelli.

EXPLANATORY. First described in 1897 by Sanarelli, and claimed by him to be the cause of yellow fever. Very closely related to preceding.

REFERENCES. Sanarelli, Ann. Past. Inst., 1897; H. 369; L. & N. 256; M. & R. 453; McF. 518; P. 609.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

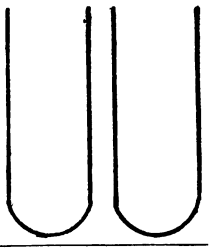
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

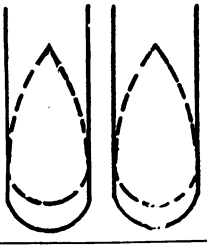
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

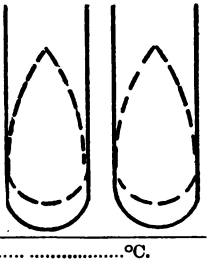
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

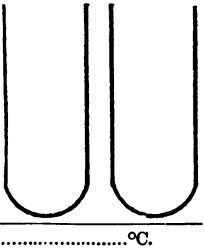
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus typhosus Zopf.

SYNONYM. Bacillus typhi abdominalis Aut.

COMMON NAMES. Typhoid bacillus; Eberth's bacillus.

EXPLANATORY. First described by Eberth in 1880, first cultivated by Gaffky, 1884. It is found in the feces 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; C. 213; Fl. 2, 384; H. 223; L. & K. 166; L. & N. 232; Mig. 727; M. & R. 317; M. & W. 141; McF. 466; P. 402; S. 337.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

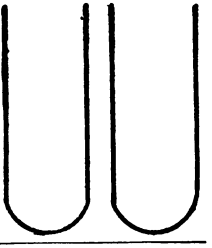
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

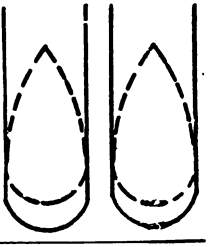
Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

Gelatin Stab: GROWN 24 HOURS AT.....°C.



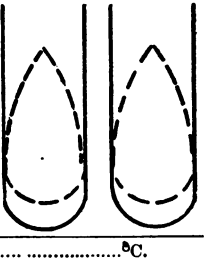
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Agar Streak: GROWN 24 HOURS AT.....°C.



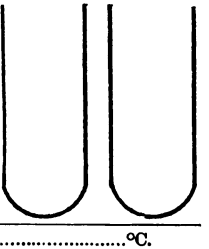
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C. | 6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2$: : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus Shigae Chester

SYNONYMS. Bacillus of Japanese dysentery, Shiga.

EXPLANATORY. First described by Shiga who found it causally related to a dysentery epidemic; Flexner has more recently found it in the Philippines and elsewhere.

REFERENCES. Shiga, C. f. B., 1898, 33, 599; C. 228; McF. 514.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

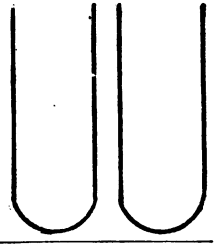
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

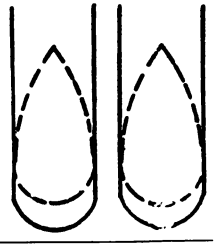
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

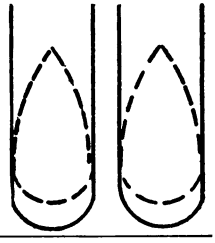
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

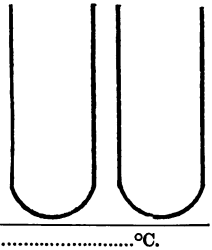
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT .....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus pestis Lehm & Neum.

SYNONYMS. Bacterium pestis Lehm & Neum; Bacillus pestis-bubonicæ Kruse.

COMMON NAME. Bacillus of bubonic plague.

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

REFERENCES. Kitasato, Lancet, 1894, 2, 428; Yersin, Ann. de l' Inst. Past., 1894, 8, 662; A. 292; C. 215; Fl. 2, 429; H. 259; L. & K. 200; M. & R. 437; L. & N. 213; Mig. 2, 749; McF. 551; P. 606.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

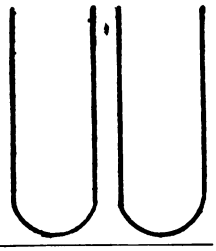
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

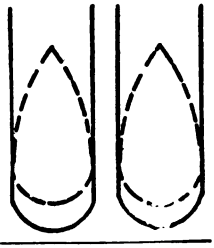
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

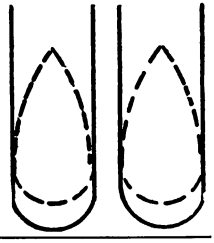
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

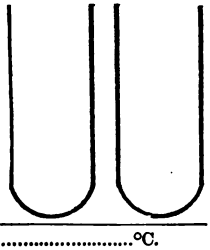
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Pseudomonas aeruginosa (Schroeter) Mig.

SYNONYM. Bacillus pyocyaneus Gessard.

COMMON NAME. Bacillus of blue-green pus.

EXPLANATORY. First described in 1872 by Schroeter. Found in green pus, and 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; C. 321; Fl. 2, 296; H. 138; L. & K. 120; L. & N. 281; Mig. 884; M. & R. 170; M. & W. 160; McF. 262; P. 535; S. 454.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

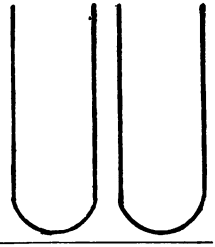
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

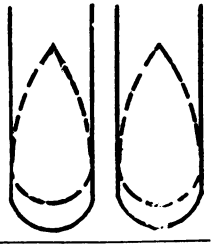
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

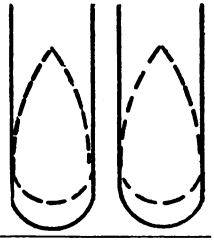
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

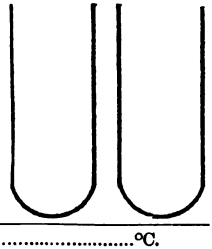
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Microspira comma (Koch) Schroeter.

SYNONYMS. Spirillum choleræ-asiaticæ Zopf; Vibrio choleræ Lehm. & Neum.

COMMON NAME. Comma bacillus; cholera bacillus.

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

REFERENCES. Koch, Berl. Klin. Wochenschr., 1884, no. 31 u. 32; A. 401; C. 335; Fl. 2, 527; H. 244; L. & K. 181; L. & N. 353; Mig. 2, 960; M. & R. 402; M. & W. 152; McF. 421; P. 568; S. 500.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

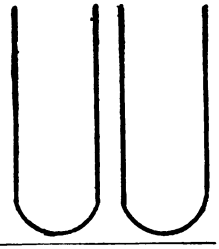
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

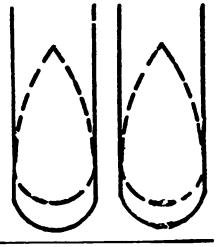
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

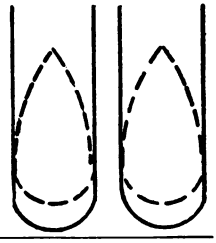
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

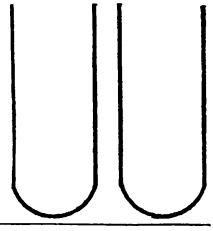
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... :

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Microspira Metschnikovi (Gamaleia) Mig.

SYNONYM. Vibrio Metschnikovi Gamaleia.

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

REFERENCES. Gamaleia, Ann. d l' Inst. Past., 1888, 2, 482; A. 441; C. 334; Fl. 2, 587; H. 256; L. & N. 366; Mig. 2, 979; M. & R. 426; McF. 442; P. 593; S. 511.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

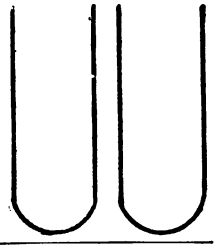
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

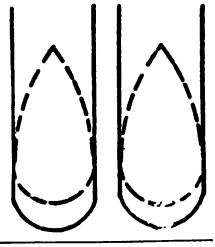
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

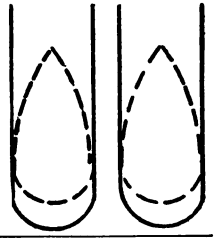
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

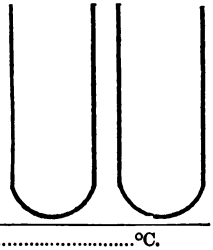
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, H : CO_2 : : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk.....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Microspira Schuylkilliensis (Abbott) Chester.

SYNONYM. Vibrio Schuylkilliensis Abbott.

EXPLANATORY. Isolated from the Schuylkill river water by Abbott in 1896. Very similar to preceding.

REFERENCES. Abbott, Jour. Exp. Med., 1896, 1, p. 419; C. 334.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

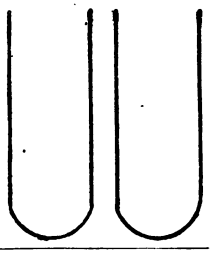
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

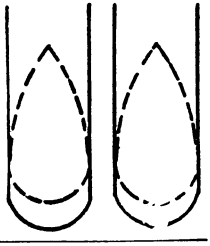
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

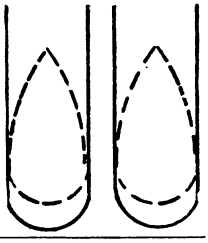
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

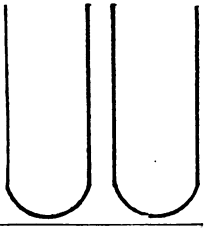
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1 Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Streptothrix bovis (Harz) Chester.

SYNONYMS. Actinomyces bovis Harz; Streptothrix Actinomyces Rossi Doria; Oospora bovis Sauv. et Radais.

COMMON NAMES. Ray fungus; Actinomyces.

EXPLANATORY. First described by Bollinger. It occurs in actinomycosis or lumpy-jaw in cattle, hogs, horses and man. It probably leads a saprophytic life on plants, etc.

REFERENCES. C. 361; Fl. 2, 51; L. & N. 440.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon..	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

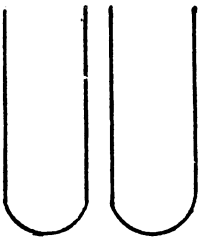
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

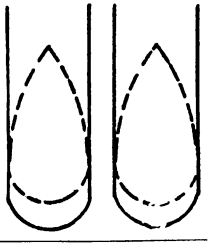
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

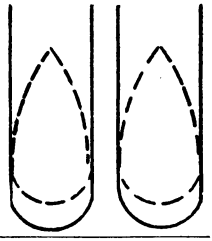
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

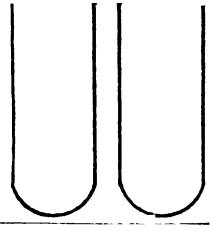
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, H : CO_2 : : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Streptothrix Madurae Vincent.

EXPLANATORY. First described by Vincent. Associated with a warty, ulcerative affection of the feet, but rarely of the hands.

REFERENCES. Vincent, Ann. Past. Inst., 1894; C. 368; Fl. 2, 58; L. & N. 452.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

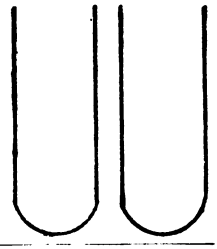
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

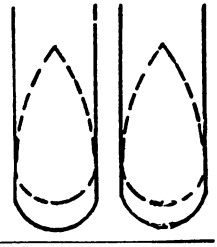
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

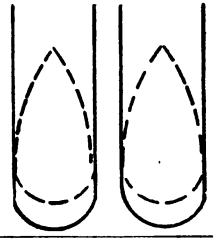
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

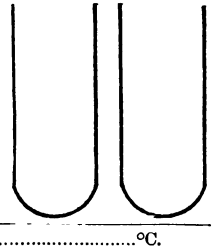
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2$: : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

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 (Fig. 27). The gas should be washed, 1st. in lead nitrate to absorb the sulphuretted hydrogen, 2d. in silver sulphate to absorb any arseniuretted or phosphuretted hydrogen, and 3d. in potassium hydrate to remove sulphur and carbon dioxide.

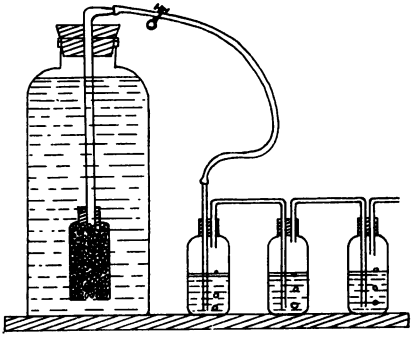


FIG 27. Hydrogen generator.

The cultures should be made in dextrose media (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 hermetically sealed. A very convenient method has recently been outlined by Wright for test-tube cultures. It is as follows: After the culture medium has been inoculated in the usual manner, thrust the cotton plug into the test-tube so that the upper end of the cotton is about 2 cm. below the mouth of the test-tube (it is usually desirable to cut off a part of the protruding portion before doing this). Fill the tube with pyrogallic acid. Add with a pipette enough of a 4% solution of sodium hydrate to dissolve the acid. Close the tube *immediately*, making it air tight by inserting a rubber stopper in its mouth. Then invert, in the case of solid media, and set aside for development. Fig. 28.

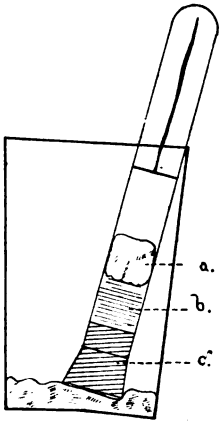


FIG. 28. Wright's method for cultivating anaerobes.

REFERENCES. A. 206; L. & K. 98; M. & R. 68; M. & W. 117; McF. 216; P. 233; S. 78. Wright, Jour. Boston Soc. of Med. Sci. 1900, 5, 114.

Bacterium Welchii Mig.

SYNONYM. Bacillus Aerogenes Capsulatus Welch.

EXPLANATORY. 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 and Flexner, Jour. Exp. Med., 1896, 1, 5; C. 183; Fl. 2, 243; H. 140; L. & N. 344; Mig. 392; M. & W. 173; McF. 585; P. 545; S. 731.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

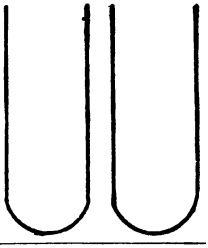
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

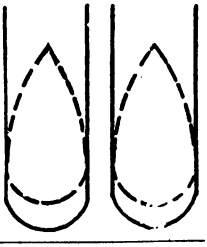
Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

Gelatin Stab: GROWN 24 HOURS AT.....°C.



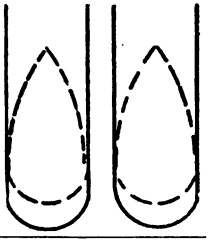
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Agar Streak: GROWN 24 HOURS AT.....°C.



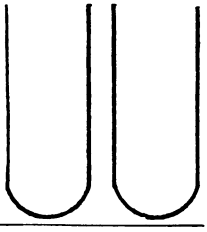
48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C. | 6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT .....°C. | 6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus Feseri (Trevisan) Kitt.

SYNONYMS. Bacillus carbonis Mig.; Bacillus anthracis-symptomatici Kruse.

COMMON NAME. Bacillus of symptomatic anthrax; Black-leg bacillus.

EXPLANATORY. 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 boeuf, 2nd edit. Paris, 1887; A. 482; C. 296; Fl. 2, 245; H. 304; L & N. 339; Mig. 593; McF. 575; P. 563; S. 493.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

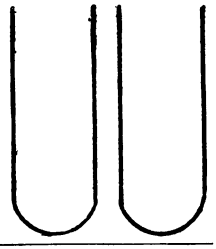
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

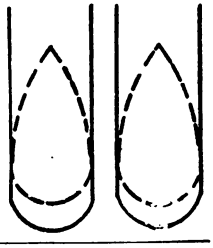
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

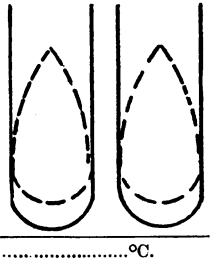
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

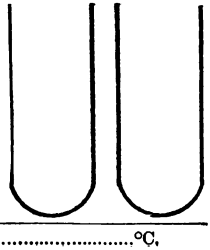
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1 Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, H : CO_2 : : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus edematis Zopf.

SYNONYM. Bacillus edematis-maligni Zopf.

COMMON NAME. Bacillus of malignant edema.

EXPLANATORY. First described by Pasteur in 1877. Widely distributed in soil and putrefying material. Few cases on record of infection in man.

REFERENCES. Zopf, Spaltpilze, 1885, 88; A. 476; C. 292; Fl. 2, 234; H. 302; L. & K. 305; L. & N. 341; Mig. 604; M. & R. 394; M. & W. 175; McF. 581; P. 543; S. 488.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

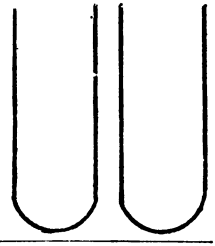
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

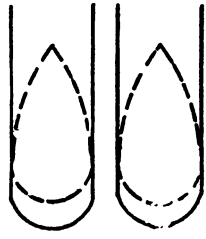
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

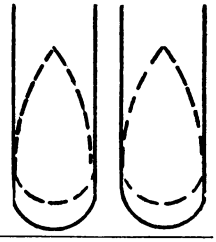
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

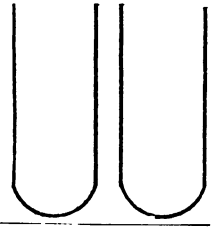
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT.....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus Botulinus v. Ermengem.

EXPLANATORY. Isolated by v. Ermengem from ham which had caused meat poisoning. Believed to be the cause of meat poisoning characterized by nervous symptoms of central origin, *botulism*.

REFERENCES. v. Ermengem, Z. f. H., 1898, 26, 1; C. 297; L. & K. 245; L. & N. 337; Mig. 616.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

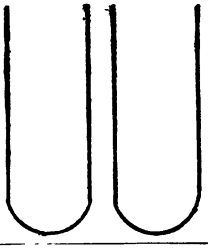
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

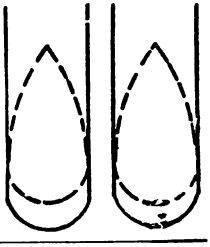
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

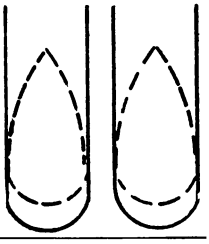
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

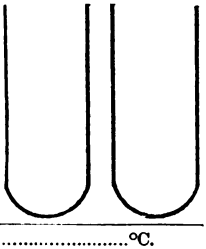
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT .....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours.....per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2$: : ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

Bacillus tetani Nicolaier.

EXPLANATORY. Discovered by Nicolaier, 1884. First cultivated by Kitasasto, 1889. Occurs in man and in animals suffering from the disease, and is widely distributed in nature, especially in soil.

REFERENCES. Nicolaier, Deutsche Med. Wochenschrift, 1884; Kitasato, Deutsche Med. Wochenschrift, 1889; A. 469; C. 302; Fl. 2, 260; H. 296; L. & K. 230; L. & N. 332; Mig. 592; M. & R. 376; M. & W. 171; McF. 371; P. 385; S. 482.

MORPHOLOGICAL CHARACTERS.	SKETCHES.
1. Form and arrangement:	
a. Bouillon	
b. Agar	
c. Gelatin	
d. Other media	
2. Size:	
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:	
a. Capsules	
b. Involution forms	
c. Deposits or vacuoles	
d. Pleomorphism	

Reaction of media (Fuller's scale) +..... or −.....

Gelatin plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

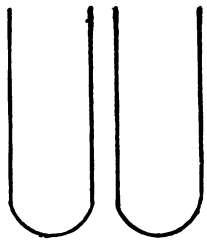
48 HOURS AT.....°C.	6 DAYS AT.....°C.

Agar plate: GROWN 24 HOURS AT.....°C.	SKETCH.
(a) Surface Colonies.	
(b) Deep Colonies.	

48 HOURS AT.....°C.	6 DAYS AT.....°C.

Special Media: (SUCH AS LITMUS MILK AND BLOOD SERUM.)

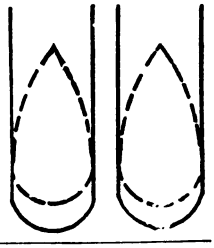
Gelatin Stab: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

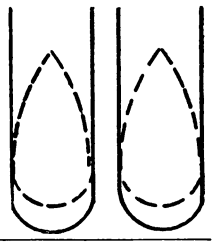
Agar Streak: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

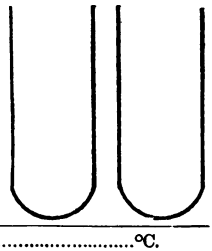
Potato: GROWN 24 HOURS AT.....°C.



48 HOURS AT.....°C.

6 DAYS AT.....°C.

Bouillon: GROWN 24 HOURS AT.....°C.



24 HOURS AT .....°C.

6 DAYS AT.....°C.

1. Relation to temperature: .....

optimum.....°C.; limits..... to.....°C.;

thermal death-point.....°C.; time of exposure.....minutes.;

medium in which exposure is made.....

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

.....

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

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

.....

.....

4. Pigment production: .....

.....

5. Gas production in sugar media: .....

a. dextrose (1) Shake culture.....

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

rate of development: 24 hours.....per cent., 48 hours..... per cent.

72 hours.....per cent., ...hours.....per cent.

reaction in open arm.....

gas formula, $H : CO_2 : :$ ..... : .....

b. lactose..... *c.* saccharose.....

6. Acid or alkali production: .....

.....

litmus milk .....

.....

7. Reduction of nitrates: .....

to nitrites..... to ammonia.....

8. Indol production: .....

48 hours.....days .....

9. Enzyme production: .....

.....

proteolytic.....

digestion of gelatin..... digestion of casein.....

diastatic.....

.....

10. Characteristic odor: .....

11. Pathogenesis (or other special characters): .....

.....

.....

.....

.....

.....

.....

CHAPTER VIII.

ANIMAL INOCULATION AND STAINING OF BACTERIA IN TISSUE.

EXERCISE 97. 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 white rats, rabbits, guinea pigs and pigeons. Inoculations are usually made subcutaneously, intraperitoneally or intravenously, 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 material injected.

b. For solid material a pocket is used which is stitched, or sealed with contractile celloidin, after the material is introduced.

Intraperitoneal. Either liquids or solid material may be introduced.

a. For liquids. The seat of inoculation is prepared as above, the syringe needle is then plunged directly into the peritoneal cavity.

b. For solid material. The animal is anesthetized; the hair is clipped or shaved from a portion of the median line, about half way between the pubis and the sternum; a slit is made in the skin with sterile instruments; the smallest possible opening is made along the linea alba into the peritoneal cavity and the material introduced; the wound closed and the body wall and the skin stitched separately. It is hardly necessary to add that the whole operation is carried out under the most strict aseptic precautions.

Celloidin Sacs. The use of the celloidin sac has recently become very common and deserves description as one of the necessary laboratory procedures. The difficulty in making these sacs has been largely overcome by the use of the gelatin capsules employed for administering medicine. Fig. 27, 1 shows such a capsule one-half natural size. A glass tube, slightly smaller in diameter than the bottom of the capsule, is first drawn out as indicated in Fig. 27, 2 and then heated and fitted into the bottom of the capsule; the hot glass melts the gelatin and causes immediate adhesion. The capsule is now coated, by alternately rolling or dipping it in thin celloidin (Fig. 23, 3.) and removing and drying it in the air. This process is continued until the coat of celloidin is about equal in thickness to that of the capsule. It is now filled with water and placed in a test-tube of water (Fig. 27, 4.). It is next heated in the steamer or autoclav, and the melted gelatin poured out when it is refilled with water or bouillon and sterilized. In this condition it can be kept until needed.

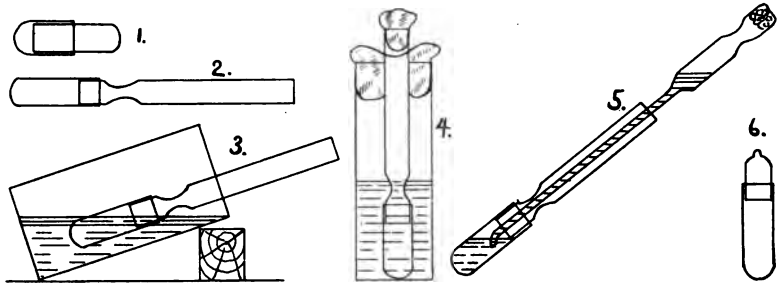


FIG. 27. Preparation of Celloidin Sac (½ natural size). 1. gelatin capsule, 2. lower part of capsule sealed on a glass tube, 3. method of coating with celloidin, 4. method of sterilizing, 5. method of filling, 6. completed capsule (Adapted from McCrae & Novy).

In the process of making it is necessary to avoid hitting the sac against the sides of the vessel. The formation of large bubbles must also be guarded against as they are likely to rupture during sterilization.

When the sac is to be used the water is drawn out by means of a sterilized Pasteur pipette (Fig. 27, 5.), and, in the same way, filled with the inoculated bouillon culture. After drying the tube at its constricted portion it is drawn out and sealed. Fig. 27, 6 shows the completed capsule. This can be kept in a sterile Petri dish. It is a good plan to wind silk thread at the place of union between glass and capsule before sterilization. The loose ends aid in the handling of the capsule.

It is desirable to test the sac by keeping it in a tube of bouillon

for 24 hours after the organism has been added. If the bouillon in the tube remains clear it is then safe to place the sac in the animal.

This method of inoculation is especially useful in increasing the virulence of attenuated forms, and in producing immunity in animals to induce the agglutinating and lysogenic properties in the blood.

REFERENCES. N. 496; McCrae, Jour. Exp. Med., 1901, 5, 635.

Intravenous. A rabbit is generally chosen for this purpose and the inoculation made into an ear vein. Of the three branches of the *vena auricularis posterior*, the *ramus lateralis posterior* is the smallest, but, due to the fact that it is the most firmly imbedded in connective tissue, it is much more easily entered than the others. The artery forceps (Fig. 28, *a*) are used to gorge the the vessel and, are of course, removed before the materia is injected. Avoid the introduction of air, which causes immediate death, and keep the animals under close observation for one hour.

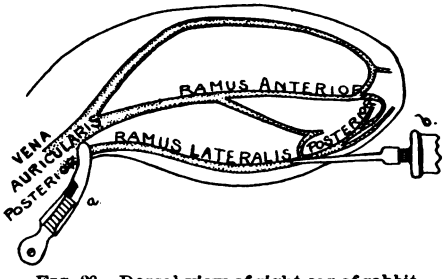


FIG. 28. Dorsal view of right ear of rabbit. *a*, artery forceps; *b*, syringe.

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 tunction with the sclerotic, the needle passing into the anterior chamber in a plane parallel to the plane of the iris.

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 benzine or alcohol and burned. This is less injurious to the instruments than heating in the direct flame.

Use blank, p. 302 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. Fig. 29 shows a simple cage made of gavanized iron with soldered seams. After use it is sterilized by boiling water in it. The wire door is covered with a cloth to prevent the too rapid escape of steam.

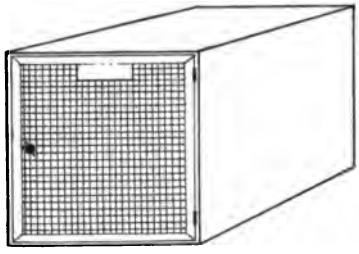


FIG. 29. Wesbrook's sterilizable, galvanized-iron animal cage.

The inoculated animals must be kept under constant observation and the following conditions noted:

a. Temperature.

b. Loss of weight.

c. Peculiar position in cage.

d. Loss of appetite.

e. Condition of the coat or hair.

f. Condition of the secretion of the air passages, conjunctiva and kidneys; diarrhea or hemorrhage from the bowels.

g. 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.

a. Inspect externally and note presence and character of any lesion.

b. 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.

c. 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.

d. 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.

a. 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.

b. 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.

c. When the thoracic and abdominal cavities are fully exposed, a careful examination of the organs and surroundings is made without disturbing them.

Plates (Petri-dishes) or roll cultures are prepared from the blood, liver, spleen, kidneys, and from 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 from 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.

Use blank on p. 302 for preservation of data. Fig. 30 shows the method of making a post-mortem and the location of the most important lymphatic glands.

REFERENCES. The above is taken largely from Bowhill, 74; see also A. 219; N. 260; and other texts.

COMMON LABORATORY EXPERIMENTS.

The following inoculations are those most frequently made:

Streptococcus erysipelatos. Mice or rabbits, *intravenous.*

Sarcina tetragena. Guinea pigs and white mice, *subcutaneous.*

Bacterium anthracis. Guinea pigs or rabbits, *subcutaneous.*

Bacterium pneumoniæ. Rabbits and mice, *subcutaneous.*

Bacterium pneumonicum. Mice and young rats, *intraperitoneal.*

Bacterium tuberculosis. Guinea pigs, rabbits and field mice, *subcutaneous* or *intraperitoneal.*

Bacterium mallei. Male guinea pigs, *intraperitoneal.*

Bacterium diphtheriæ. Guinea pigs, rabbits and fowl, *subcutaneous* and *intratracheal.*

Bacillus pestis. Rats, mice, guinea pigs and rabbits, *subcutaneous.*

Bacillus Salmoni. Rabbits and mice, *subcutaneous.*

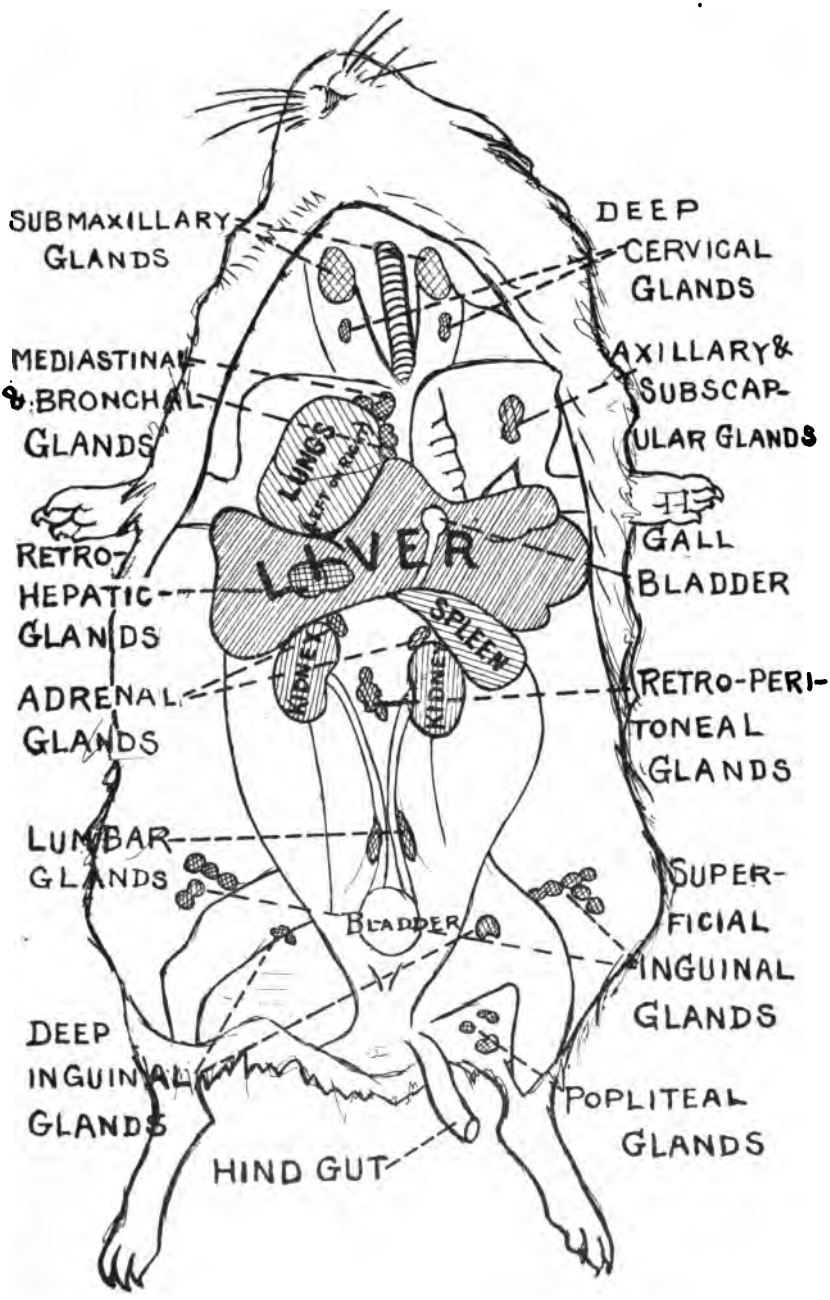


FIG. 30. Diagram showing method of making autopsy on guinea pig; and also the most important glands (adapted from Delapine & Curtis).

BLANK FOR ANIMAL EXPERIMENTS.

Animal .....No.....Sex..... Age.....Weight.....

Date.....o'clock.....M.

Inoculated with.....

How inoculated.....

....

Symptoms produced:

Died (or killed) ....o'clock.....M.

Autopsy made.....o'clock.....M.

Autopsy Findings:

Bacteriological Examination:

Histological Examination:

Organs preserved..... .....

Museum No. .....**Slide Nos.** .....

EXERCISE 98. 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 hours. 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.	B.	C.
Paraffin Method.	*Celloidin Method.*	*Freezing Method.*
a. Absolute alcohol 6–24 hours.	*a.* Mixture of ether and absolute alcohol (equal parts) 24 hours.	*a.* Place in 1% formalin 2 hours.
b. Xylene 6–24 hours.	*b.* Thin celloidin (about 6%) 24 hours to several weeks.	*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.
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.	*c.* Thick celloidin (about 12%) 24 hours to several weeks.	
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.	*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.	

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 99. STAINING SECTIONS.

GENERAL HISTOLOGICAL METHOD.

Hematoxylin and Eosin.

a. Transfer sections from alcohol to distilled water.

b. Stain in alum-hematoxylin 5 minutes. The stain may be prepared as follows (Boehmer):

1. Hematoxylin 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. Acid alcohol 5 to 10 seconds.

d. Ammonia water (1½%) until sections are a light blue.

e. Wash in water.

f. Counter-stain with eosin ($\frac{1}{10}$% to ½% in 60% alcohol) 3 minutes.

g. Alcohol, 95%, two or three changes to dehydrate and remove excess of counter-stain.

h. Clear in oil of origanum, or in Dunham's mixture (white oil of thyme 4 parts, oil of cloves 1 part).

i. Balsam.

GENERAL BACTERIOLOGICAL METHODS.

A. ***Loeffler's Universal Method.***

a. Take sections out of alcohol and place in Loeffler's methylen blue for 5 to 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. Carmine, 5 minutes.

b. Acid alcohol, 15 seconds.

c. Wash in water.

d. Transfer to slide and blot.

e. Ehrlich's anilin water gentian violet 3 minutes.

f. Blot.

g. Place in potasium iodide and iodine solution (iodine 1 part, potassium iodide 2 parts, water 100 parts) 2 minutes.

h. Blot.

i. Decolorize in a mixture of anilin oil 2 parts and xylene 1 part, 2 to 5 minutes.

j. Blot.

k. Mount in balsam.

This stain can only be used with those organisms which take the Gram stain, namely: ***Str. erysipelatos, M. pyogenes var. albus, M. pyogenes var. aureus, Sar. tetragena, Bact. anthracis, Bact. pneumoniæ Bact. rhusiopathiæ, Bact. tuberculosis, Bact. leprœ, Bact. diphtheriæ, Ps. aeruginosa, Bact. Welchii, B. Feseri, B. edematis, B. tetani and Streptothrix bovis.***

SPECIAL BACTERIOLOGICAL METHODS.

Particular organisms may be stained as follows:

Pus micrococci. Loeffler's or Weigert's method.

Micrococcus gonorrhoeæ. Loeffler's method gives the best results.

Sarcina tetragena. Loeffler's or Weigert's method.

Bacterium anthracis. Loeffler's or Weigert's method.

Bacterium pneumoniæ. 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 of gentian violet: -	50 cc.
Distilled water - - - - - - - -	100 cc.
Glacial acetic acid - - - - - - - -	10 cc.

b. Wash out in 1% solution of acetic acid.
c. Alcohol.
d. Xylene.
e. Canada balsam.

Bacterium choleræ. Loeffler's method.

Bacterium tuberculosis.

a. Weigert's method (staining with anilin oil gentian violet 24 hours at room temperature, or 2 to 3 hours at 40° C.).

b. Ziehl-Neelsen's Method.

1. Stain with carbol-fuchsin (12 to 24 hrs. room temperature, 1 to 3 hrs. 40° C.)
2. Decolorize with nitric acid (10%) a few seconds, and then with alcohol (60 to 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 lepræ.

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 to 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 to 8 hours.
b. Wash in distilled water.
c. Tannic acid solution (10 %) 4 to 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 to 30 seconds.
b. Wash in distilled water.
c. Tannic acid solution (10%) ½ to 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 diphtheriæ. Loeffler's or better Weigert's method.

Bacillus typhosus.

a. Loeffler's methylen blue or carbol-fuchsin 15 min. to 24 hrs.
b. Wash slightly in distilled water.
c. Place in 30% solution of tannic acid for 10 to 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 Salmonii. Loeffler's method.

Bacterium Welchii. Weigert's and Loeffler's methods.

Bacillus Feseri. Use Pfeiffer's stain:

a. Dilute carbol-fuchsin ½ hour.
b. Absolute alcohol slightly acidulated with acetic acid until section is a reddish violet tint.
c. Xylene and examine.
d. Mount in balsam.

Bacillus edematis. Pfeiffer's stain.

Streptothrix bovis.

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 100. 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 by forceps. This material may be examined directly by means of cover-glass preparations or by means of cultures.

a. 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 Loeffler's Blood serum) is placed in a box for transportation. FIG. 31.

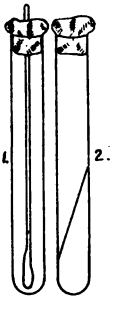


FIG. 31. Diphtheria Outfit.

b. 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.

ORGANISMS COMMONLY FOUND.

Bacterium Diphtheriæ.

The presence of this germ in the mouth usually results in the formation of a pseudo-membrane, a portion of which is to be removed with a pair of forceps, or by means of the outfit described above. It should be examined directly for the diphtheria bacillus by smearing on a cover-glass and staining 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 bismarck brown dissolved in a liter of distilled water. Films are stained in *a.* 5 to 8 seconds, washed in water, stained in *b.* 3 to 5 seconds, dried and mounted.

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 to 38° C. for 12 to 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. diphtheriæ	B. pseudo diphtheriticum
1) Form	Slender, and of same diameter throughout	Thicker at center than ends, plumper shorter and less variable than B. diphtheriæ
2) Size	Average 1.2 to 2 μ	Averaging 1 to 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

Pus Micrococci. (Str. erysipelatos, M. progenes var. aureus and albus, Sar. tetragena.)

a. Stained cover-glass preparations are to be examined, and if micrococci are found make:

b. 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:

a. Under the microscope in a drop of glycerine.

b. Cover-glass preparations stained with carbol-fuchsin or Gram's method.

c. 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; Si. 101. See also various texts under special organism.

EXERCISE 101. 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.

ORGANISMS MOST COMMONLY FOUND.

Bacterium Tuberculosis. Place the sputum in a Petri dish over a black surface and select one of the little cheesy masses, if these be 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:

a. Gabbett, see Part 1, p. 58.

b. Ziehl-Neelsen:

1. Carbol-fuchsin ten times through the flame (5 to 10 min.)
2. Nitric acid (30%) momentarily.
3. Water.
4. Alcohol (60%) until red color disappears. It may be necessary to immerse preparation in acid a second time, but care must be exercised to prevent extraction of dye from tubercle bacterium.

5. Loeffler's methylen blue, 1 minute.
6. Mount and examine.

While the tubercle bacteria may be detected when present in considerable numbers with a $\frac{1}{6}$-inch objective, when there are few present, a $\frac{1}{12}$-inch oil immersion will be necessary, and this ought to be used to search all slides where the tubercule 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 mucous, 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:

a. Add 5% of crystallized carbolic acid (in the case of sputum add 5 times its bulk of a 5% solution of carbolic acid).

b. Place 15 cc. in the tubes of a centrifuge and whirl for 15 minutes.

c. Pour off supernatant fluid and treat precipitate with 3 cc. of a 5% KOH solution. Mix thoroughly and allow to stand 2 minutes.

d. Fill to 15 cc. mark with distilled water and whirl 20 minutes.

e. 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 a 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 influenzæ. 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 pneumoniæ.

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 **27.**)

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 is most susceptible and should be inoculated subcutaneously. As a result of infection with this organism the animal quickly dies with a typical septicemia, 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 **105.**

Streptothrix bovis. This organism has been occasionally found in sputum and in such cases the peculiar morphology of the colonies is well brough out by Gram's method. See **105.**

REFERENCES. v. J. 114; Si. 245. See also various texts under particular organisms.

EXERCISE 102. EXAMINATION OF BLOOD.

COLLECTION.

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 microscopical 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 place a second cover-glass on this; this flattens the drop of blood out into a thin film. Immediately and before coagulation 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:

a. Microscopical examinations of blood or of 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.

b. In hanging drop preparation large, homogeneous, immotile bacilli.

c. Agar plate cultures should also be made, and, from the separate colonies, subcultures; the gelatin stab being especially characteristic.

d. In important cases (as in man) guinea pigs, or white mice, should be inoculated, and, in case of death, organism isolated and identified.

Spirochaeta 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). It has a brisk, vibratile movement in the direction of its long axis, and is very sensitive to reagents of all kinds. Even the addition of distilled water will cause it 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 actic 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.

Pus Micrococci. These are occasionally found, and for method of detection see **105.**

Bacterium mallei. Sometimes found in the blood of those suffering with Glanders. It may be detected in the blood-smears. For special methods see **105.**

Bacterium pneumoniæ. 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.

Bacterium tuberculosis. In case of military tuberculosis they may be very rarely found in sufficient numbers to be detected by staining methods, see sputum **101.**

Bacterium influenzæ. Canon claims to have stained and cultivated this organism in blood, but this needs confirmation.

Bacillus coli. This organism may be found in the blood. For methods of isolation and identification see Feces **103.**

Bacillus pestis. This germ occurs in the blood, in certain cases at least. Consierdable skill in detecting it is required—due to its variable appearance. Broth tubes should be infected and animals inoculated.

Bacillus Salmonii.

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 from 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 hemoglobin, 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).

REFERENCES. v. J. 45: Si. 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 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 to 72 hour old agar, or bouillon, culture of *Bacillus typhosus.*

b. If the bacilli be 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.

The dilution is made in the following way: Nine drops of sterile water are placed around the drop of dried blood. (The drops of water should be of about the same size as that of the original drop of blood.) The drops are all mixed together and allowed to soak up the blood. In this way an approximate dilution of one to ten is obtained. One drop of this is added to the hanging-drop culture. This gives a dilution of one to twenty which is the one usually employed.

Exact dilutions may be made by weighing out the blood and adding it to a measured amount of water.

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." The usual time limit is thirty minutes.

REFERENCES. v. J. 45; Si. 79. See also texts under particular organism.

EXERCISE 103. EXAMINATION OF FECES.

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 occurring here is enormous, and comprise a large number of species and among them several pathogenic forms particularly *B. typhosus, Msp. comma, Bact. tuberculosis* and *Amoeba coli.*

Bacillus typhosus. This organism occurs in the feces 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 servicable:

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 num-

ber 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 feces 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 their agglutinating power on typhoid blood. Instead of the use of the lactose litmus agar plate, either Elsner's or Hiss' methods may 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 pototoes 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 (10cc. 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 dextrose.
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 2% normal acid, cleared with two eggs and filtered, dextrose 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 larger 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 dextrose.
1000 grams water.

Made as plate medium except that it 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 microscopically 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.

Ameba coli.

a. 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 ameboid movements.

b. Preparations may be stained with methylen blue and carmine. The nucleus is stained with the carmine.

c. Discharges may be hardened and stained by Mallory's method as follows:

1. Fix tissues in alcohol.
2. Stain (paraffin) sections in a saturated aqueous solution of thionin for 5–20 minutes.
3. Wash in water.
4. Differentiate in a 2% aqueous solution of oxalic acid ½–1 minute.
5. Wash in water.
6. Dehydrate in alcohol (95%).
7. Clear in oil of bergamot.
8. Wash with xylene and mount in balsam.

Nuclei of Amebae brownish red, other nuclei blue.

REFERENCES. v. J. 199; Si. 228. See also texts under various organisms.

EXERCISE 104. 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, **103.**

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.):

a. Absolute alcohol, 3 hours.
b. Chromic acid, 15 minutes.
c. Stain in hot carbol-fuchsin.
d. Decolorize in sulphuric acid (25%) 2–3 minutes.
e. 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.

Pus Micrococci. **105.**
Micrococcus gonorrhœæ. **105.**
Bacillus typhosus. **103.**
Spirochaeta Obermeieri. **102.**

REFERENCES. v. J. 273; Si. 504, and texts under the various organisms.

EXERCISE 105. 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 Pus Coccus Group.

b. If streptococci suspect Str. erysipelatos.

c. If diplococci or tetracocci.

1. Within the pus-cells test for M. gonorrhoeae or M. Weichselbaumii.
2. Free suspect Sar. 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. Bact. anthracis. 3. Bact. pneumoniæ. 4. Bact. tuberculosis. 5. Bact. lepræ. 6. Bact. mallei. 7. B. pestis. 8. Ps. æruginosa. 9. Bact. Welchii. 10. B. edematis. 11. B. tetani.

e. Streptothrix bovis.

f. Ameba coli.

Pus Micrococci. These organism are frequently present in pus and should be isolated and identified in pure cultures, as microscopical examinations alone, will not suffice.

Streptococcus erysipelatos. This organism is not infrequently present and can be readily identified by culture methods.

Micrococcus gonorrhœæ. Pus should be collected in a sterile receptacle or spread on cover-glasses and allowed to dry. When once dried it should not be wet or moistened again as this would destroy the pus-cells, and hence the value of the material for diagnosis.

a. Simple stain.

1. Loeffler's methylen blue 3–5 minutes.
2. Wash in water.
3. Dry, mount in balsam and examine with $\frac{1}{12}$ in. oil immersion.
4. Look for a biscuit-shaped diplococcus within the pus cells.

b. Gram's method.

1. Anilin oil gentian violet 15 minutes.
2. Wash in water.
3. Treat with iodine solution 2 minutes.
4. Decolorize with alcohol.

5. Counter-stain with Bismarck brown, ¾ minutes.
6. Wash, dry and mount in balsam.
7. Examine with oil immersion.

If the gonococci be present they will be stained brown.

If diagnosis be of great importance make cultures as follows:

a. Make 6 or more streak cultures on blood agar, or better, make plates on Wertheim's medium (p. 154). Grow at 38° C.

b. 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.

Micrococcus Weichselbaumii.

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 on 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 Weichselbaumii 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.

Bacillus coli. **103.**

Bacterium tuberculosis. **101.**

Bacterium leprœ. For method of staining, see **99.**

Bacterium pneumoniæ. Stain for capsule. Cultivate on blood-agar. **101.**

Bacterium 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.

Bacillus 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.

Pseudomonas aeruginosa. Easily recognized by its culture characters.

Bacterium Welchii.

This germ is non-pathogenic for rabbits, but Welch and Flexner have shown that if a rabbit be 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.

Bacillus edematis.

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.

Bacillus tetani.

a. Make cover-glass preparation from pus and search for drumstick bacillus.

b. Make dextrose bouillon and agar-plate cultures and develop in hydrogen.

c. Inoculate animals with the discharge, and also with the bouillon culture, and watch for characteristic symptoms.

Streptothrix bovis.

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.

Ameba coli. **103.**

REFERENCES. v. J. 405; Si. 514 and 518. See also texts under the various organisms.

EXERCISE 106. DIAGNOSIS OF RABIES.

A. Pasteur's Method.

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. Anesthetize 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 hemorrhage 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, 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.

B. Van Gehucten and Nélis' Method.

a. The head of the dog (shipped as directed above) is dissected and the ganglion on the pneumogastric nerve is selected by preference (Fig. 32, A.)

b. The ganglion is treated as follows:

1. Absolute alcohol 6 hours, then in fresh alcohol 6 hours.
2. Xylene 6 hours.
3. Imbedded in paraffin.
4. Sectioned.

Or 1. 10% formalin 6 hours.
2. 95% alcohol 6 hours.
3. Absolute alcohol 6 hours.
4. Imbedded in celloidin and sectioned, or cut on a freezing microtome.

c. Stain by hematoxylin and eosin method, p. 306.

"These changes are found in the peripheral ganglia of the cerebro-spinal and sympathetic systems, and are especially marked in the plexiform ganglion of the pneumogastric nerve and the gasserian ganglion. Normally, these ganglia are composed of a supporting tissue holding in its meshes the nerve cells, each one of which is enclosed in a capsule, made up of a single layer of endothelial cells (Fig. 32, B.). The action of the rabic virus seems to exercise its effect on these cells, particularly, bringing about an abundant multiplication of the cells forming this capsule, leading finally to the complete destruction of the normal ganglion cell and leaving in its place a collection of round cells (Fig. 32 C.). Ordinarily a considerable number of ganglion cells will be found which have undergone only a slight change, but under certain conditions the process is so widespread that all the ganglion cells are destroyed. The intensity of these changes varies in different animals; they are perhaps most pronounced in the dog, less marked in man and still less in the rabbit."—Ravenel.

REFERENCES. Jour. Comp. Path. and Thera., 1901, 14, 37; Ravenel, Bull. 79 Penn. Dept. of Agri. 1901; Bailey, Jour. Exp. Med., 1991, 5, 549.

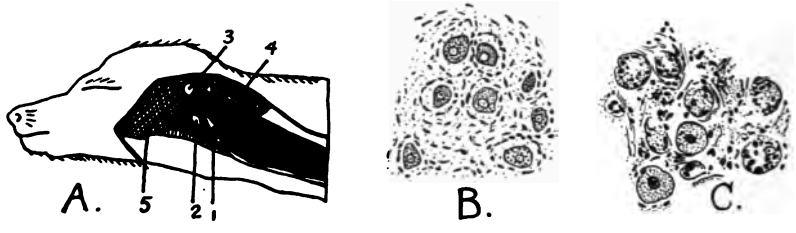


FIG. 32. Illustrating Method of Rapid Diagnosis of Rabies. A. Dissection of upper neck of Dog (After Vallé). 1 Plexiform ganglion; 2, cervical ganglion. B. Section of normal ganglion of dog. (After Crocq. Jnl. de Neurologie, V; 13.) C. section of plexiform ganglion of rabbit dead of rabies, capsules filled, or partially filled, with new formed cells (after Ravenel).

EXERCISE 107. 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. 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 108. 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 to use the slime on the filter to make the plates.

a. Parietti's method, see **103.**

b. Hiss' method. Make plate cultures and incubate at 38° C. for 18 hours. Inoculate suspicious colonies into Hiss' tube medium, fermentation tube and milk. Also make indol test and 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 be 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.

Bacterium anthracis (Robert's Method).

a. Heat suspected water to 80° C. for ten minutes to kill water bacteria.

b. Make plates in agar and in gelatin and work up colonies.

c. Inoculate a guinea pig with several cubic centimeters of the water.

EXERCISE 109. EXAMINATION OF MILK FOR PATHOGENIC BACTERIA.

Bacterium diphtheriæ.

Where *Bacterium diphtheriæ* is suspected in milk, make a considerable number of streak cultures on Loeffler's blood serum and incubate at 38° C. from 8 to 12 hours, stain and examine microscopically.

Bacterium tuberculosis.

Hammond's method of examining milk for B. tuberculosis. See Sputum, **101.**

Animal Inoculation.

USE OF MAIL FOR TRANSMISSION OF BACTERIA.

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 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."

INDEX.