(8) Cover with Van Gieson's methylene-blue-fuchsin stain, warming gently for one-half to two minutes. This stain, as modified by Frothingham, is as follows. It must be freshly mixed each day:

Tap-water	20 c.c.
Saturated alcoholic solution basic fuchsin	3 drops.
Saturated aqueous solution methylene-blue	1 drop.

(9) Wash in water and dry with filter-paper. Examine with a low power to locate the large cells in which the bodies are apt to be found, and study these with an oil-immersion lens.

The Negri bodies are stained a pale pink to purplish red, and frequently contain small blue dots (Plate XIII). The nerve-cells are blue, and red blood-corpuscles are colorless or yellowish-copper colored.

When the work is finished, the board with the dissected brain is sterilized in the steam sterilizer.

Demonstration of Negri bodies by this method is quicker and, probably, more certain than by the study of sections. It has the decided advantage over the smear method that the histologic structure is retained. One or more of the impressions generally shows the entire cell arrangement almost as well as in sections, and it is very easy to locate favorable fields with a 16 mm. objective.

CHAPTER VIII

BACTERIOLOGIC METHODS

BACTERIOLOGY has become so important a part of medicine that some knowledge of bacteriologic methods is imperative for the present-day practitioner. It has been the plan of this book to describe the various bacteria and bacteriologic methods with the subjects to which they seemed to be particularly related. The tubercle bacillus and its detection, for example, are described in the chapters upon Sputum and Urine; blood-cultures are discussed in the chapter upon Blood. There are, however, certain methods, notably the preparation of media and the study of bacteria by cultures, which do not come within the scope of any previous section, and an outline of these is given in the present chapter.

I. APPARATUS

Much of the apparatus of the clinical laboratory is called into use. Only the following need special mention:

1. **Sterilizers.**—Two are required.

The *dry*, or *hot-air sterilizer*, is a double-walled oven similar to the detached ovens used with gas and gasolene stoves. It has a hole in the top for a perforated cork with thermometer.

The *steam sterilizer* is preferably of the Arnold type, opening either at the top or the side. An *autoclave*, which

sterilizes with steam under pressure, is very desirable, but not necessary.

2. **Incubator.**—This is the most expensive piece of apparatus which will be needed. It is made of copper, and has usually both a water- and an air-jacket surrounding the incubating chamber. It is provided with thermometer, thermo-regulator, and some source of heat, usually a Koch safety Bunsen burner. With a little ingenuity one can rig up a drawer or a small box, in which a fairly constant temperature can be maintained by means of an electric light. The degree of heat can be regulated by moving the drawer in or out, or holes can be made in which corks may be inserted and removed as needed. A Thermos bottle has been suggested as a temporary make-shift.

3. **Culture-tubes and Flasks.**—For most work ordinary test-tubes, 5 by $\frac{3}{4}$ inches, are satisfactory. For special purposes a few 3 by $\frac{1}{2}$ inch and 6 by $\frac{3}{4}$ inch tubes may be needed. Heavy tubes, which do not easily break, can be obtained, and are especially desirable when tubes are cleaned by an untrained assistant.

Flasks of various sizes are needed. The Ehrlenmeyer type is best. Quart and pint milk bottles and 2-ounce wide-mouthed bottles will answer for most purposes.

4. **Platinum Wires.**—At least two of these are needed. Each consists of a piece of platinum wire about 8 cm. long, fixed in the end of a glass or metal rod. One is made of about 22 gage wire and its end is curled into a loop 1 to 2 mm. in diameter. A loop 1 mm. in diameter is sometimes called a "normal." The other wire is somewhat heavier and its tip is hammered flat.

5. **Pipets, etc.**—In addition to the graduated pipets with which every laboratory is supplied, there are a number of forms which are generally made from glass tubing as needed. One of the simplest of these is made as follows: A section of glass tubing, about 12 cm. long and 8 mm. in diameter, is grasped at the ends, and its center is heated in a concentrated flame. A blast-lamp is best, but a Bunsen burner will usually answer, par-

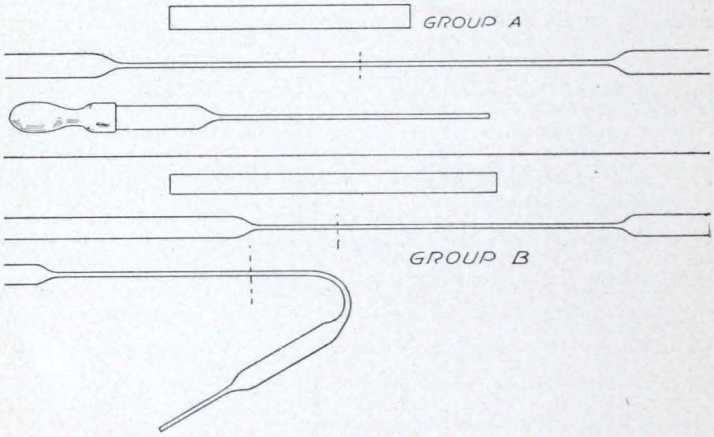

Fig. 161.—Process of making pipets (group A) and Wright's capsule (group B). The dotted lines indicate where the glass is to be broken.

ticularly if fitted with a "wing" attachment. When the glass is thoroughly softened it is removed from the flame, and, with a steady, but not rapid pull, is drawn out as shown in Fig. 161. The slender portion is scratched near the middle with a file and is broken to make two pipets, which are then fitted with rubber nipples. Two conditions are essential to success: the glass must be thoroughly softened and it must be removed from the flame before beginning to pull.

A nipple can be made of a short piece of rubber tubing, one end of which is plugged with a glass bead.

This pipet has many uses about the laboratory. When first made it is sterile and may be used to transfer cultures. With a grease-pencil mark about 2 cm. from its tip (Fig. 163), it is useful for measuring very small quantities of fluid, as in making dilutions for the Widal test and in counting bacteria in vaccines. Mett's tubes for pepsin estimation may be made from the capillary portion. The capillary portion also makes a very satisfactory blood-lancet if heated in a low flame and drawn out quickly.

Another useful device is the Wright capsule, which is made as shown in Fig. 161. Its use is illustrated in Fig. 100. After the straight end is sealed the curved portion may be hooked over the aluminum tube of the centrifuge, and the contained blood or other fluid sedimented; but the speed should not be so great as to break the capsule.

II. STERILIZATION

All apparatus and materials used in bacteriologic work must be sterilized before use.

Glassware, metal, etc., are heated in the hot-air sterilizer at 150° to 180° C. for half an hour. Flasks, bottles, and tubes are plugged with cotton before heating. Petri dishes may be wrapped in paper in sets of three. Pipets and glass and metal hypodermic syringes are placed in cotton-stoppered test-tubes.

Culture-media and other fluids must be sterilized by steam. Exposure in an autoclave to a temperature of 110° C. (6 pounds pressure) for one-half hour is sufficient. With the Arnold sterilizer, which is more commonly

used, the intermittent plan must be adopted, since steam at ordinary pressure will not kill spores. This consists in steaming for thirty to forty-five minutes on three or four successive days. Spores which are not destroyed upon the first day develop into the vegetative form and are destroyed at the next heating. Gelatin media must not be exposed to steam for more than twenty minutes at a time, and must then be removed from the sterilizer and cooled in cold water, otherwise the gelatin may lose its power to solidify.

Cotton and **gauze** are sterilized by either hot air or steam, preferably the latter.

III. PREPARATION OF CULTURE-TUBES

New tubes should be washed in a very dilute solution of nitric acid, rinsed in clear water, and allowed to drain dry.

Tubes which contain dried culture-media are cleaned with a test-tube brush after boiling in a strong solution of washing-soda. They are then rinsed successively in clear water, acidulated water, and clear water, and allowed to drain.

The tubes are now ready to be plugged with raw cotton —the "cotton batting" of the dry goods stores. This is done by pushing a wad of cotton into each tube to a depth of about 3 cm. with a glass rod. The plugs should fit snugly, but not too tightly, and should project from the tube sufficiently to be readily grasped by the fingers. The tubes are next placed in wire baskets and heated in an oven for about one-half hour at 150° C. in order to mold the stoppers to the shape of the tubes. The heating should not char the cotton, although a slight brown-

ing does no harm. The tubes are now ready to be filled with culture-media.

IV. CULTURE-MEDIA

For a careful study of bacteria a great variety of culture-media is required, but only a few—bouillon, agar or solidified blood-serum, and gelatin—are much used in routine work.

Preparation of Culture-media.—

Beef Infusion

Hamburger steak, lean	500 grams.
Tap-water	1000 c.c.

Mix well; let soak about twenty-four hours in an ice-chest, and squeeze through cheese-cloth. This infusion is not used by itself, but forms the basis for various media. "Double strength" infusion, used in making agar-agar, requires equal parts of the meat and water.

Infusion Bouillon

Beef infusion	1000 c.c.
Peptone (Witte)	10 grams.
Salt	5 "

Boil until dissolved; bring to original bulk with water; adjust reaction; and filter.

Beef Extract Bouillon

Liebig's extract of beef	3 grams.
Peptone	10 "
Salt	5 "
Tap-water	1000 c.c.

When all ingredients are dissolved, cool, and beat in the whites of two eggs; boil briskly for five minutes and filter. It is not usually necessary to adjust the reaction.

Agar-agar

Preparation of this medium usually gives the student much trouble. There should be no difficulty if the directions are carefully carried out.

Agar-agar, powdered or in shreds	15 grams.
Tap-water	500 c.c.

Boil until thoroughly dissolved and add—

Peptone	10 grams
Salt	5 "

When these have dissolved, replace the water lost in boiling, cool to about 60° C., and add 500 c.c. double-strength beef infusion. Bring slowly to the boil, adjusting the reaction meanwhile, and boil for at least five minutes. Filter while *hot* through a moderately thick layer of absorbent cotton wet with *hot* water in a *hot* funnel. A piece of coarse wire gauze should be placed in the funnel underneath the cotton to give a larger filtering surface. This medium will be clear enough for ordinary work. If an especially clear agar is desired, it can be filtered through paper in an Arnold sterilizer.

Agar can also be made by boiling 15 grams of powdered agar in 1000 c.c. of bouillon until dissolved, replacing the water lost in boiling, and filtering through paper in a sterilizer. It can be cleared with egg if desired.

Glycerin Agar-agar

To 1000 c.c. melted agar add 60 to 70 c.c. glycerin.

Gelatin

Dissolve 100 to 120 grams "golden seal" gelatin in 1000 c.c. nutrient bouillon with as little heat as possible,

adjust the reaction, cool, beat in the whites of two eggs, boil, and filter hot through filter-paper wet with hot water. Sterilize in an Arnold sterilizer for twenty minutes upon three successive days and cool in cold water after each heating.

Sugar Media

Any desired sugar may be added to bouillon, agar, or gelatin in proportion of 10 grams to the liter. Dextrose is most frequently required. When other sugars are added, media made from beef-extract should be used, since those made from beef-infusion contain enough dextrose to cause confusion.

Löffler's Blood-serum

1 per cent. dextrose-bouillon	1 part.
Blood-serum	3 parts.

Mix and tube. Place in an inspissator at the proper slant for three to six hours at 80° to 90° C. When firmly coagulated, sterilize in the usual way. A large "double-cooker" makes a satisfactory inspissator. The tubes are placed in the inner compartment at the proper slant, a lid with perforation for a thermometer is applied, and the whole is weighted down in the water of the outer compartment.

Blood-serum is obtained as follows: Beef or pig blood is collected in a bucket at the slaughter-house and placed in an ice-chest until coagulated. The clot is then gently loosened from the wall of the vessel. After about twenty-four hours the serum will have separated nicely and can be siphoned off. It is then stored in bottles with a little chloroform until needed. Red cells, if abundant, darken the medium, but do no harm.

Solidified blood-serum is probably the most satisfactory medium for general purposes. Nearly all pathogenic organisms grow well upon it.

Hemoglobin Medium

The simplest way to prepare this is to smear a drop of blood, obtained by puncture of the finger, over the surface of an agar-slant, and to incubate over night to make sure of sterility. It is used for growing the influenza bacillus.

Litmus Milk

Fresh milk is steamed in an Arnold sterilizer for half an hour, and placed in the ice-chest over night. The milk is siphoned off from beneath the cream, and sufficient aqueous solution of litmus or, preferably, azolitmin is added to give a blue-violet color. It is then tubed and sterilized.

Potato

Cylinders about one-half inch thick are cut from potato and split obliquely. These are soaked over night in running water and placed in large tubes, in the bottom of which is placed a little cotton saturated with water. They are sterilized for somewhat longer periods than ordinary media.

Dunham's Peptone Solution

Peptone	10 grams.
Salt	5 "
Water	1000 c.c.

Dissolve by boiling; filter, tube, and sterilize.

This medium is used to determine indol production. To a twenty-four- to forty-eight-hour-old culture is

added 5 to 10 drops of concentrated c. p. sulphuric acid and 1 c.c. of 1 : 10,000 solution of sodium nitrite. Appearance of a pink color shows the presence of indol. A pink color before the nitrite is added shows the presence of both indol and nitrites.

Hiss' Serum Media

Blood-serum	1 part.
Water	3 parts.

Warm and adjust reaction to + 0.2 to + 0.8. Add litmus or azolitmin solution to give a blue-violet color. Finally, add 1 per cent. of inulin or any desired sugar. The inulin medium is very useful in distinguishing between the pneumococcus and streptococcus.

Bile Medium

Ox- or pig-bile is obtained at the slaughter-house, tubed, and sterilized. This is used especially for growing typhoid bacilli from the blood during life. The following is probably as satisfactory as fresh bile and is more convenient:

Inspissated ox-bile (Merck)	30.0 grams.
Peptone	2.5 "
Water	250.0 c.c.

Dissolve, place in tubes, and sterilize.

Reaction of Media.—The chemic reaction of the medium exerts a marked influence upon the growth of bacteria. It is adjusted after all ingredients are dissolved by adding sufficient caustic soda solution to overcome the acidity of the meat and other substances used. In general, the most favorable reaction lies between the neutral points of litmus and phenolphtha-

lein, representing a very faint alkalinity to litmus. In routine work it is usually sufficient to test with litmus-paper. When greater accuracy is demanded, the following method should be used: After all ingredients are dissolved and the loss during boiling has been replaced with water, 10 c.c. of the medium are transferred to an evaporating dish, diluted with 40 c.c. of water, and boiled for three minutes to drive off carbon dioxid. One c.c. of 0.5 per cent. alcoholic solution of phenolphthalein is then added, and decinormal sodium hydroxid solution is run in from a buret until the neutral point is reached, indicated by the appearance of a permanent pink color. The number of cubic centimeters of decinormal solution required to bring this color indicates the number of cubic centimeters of *normal* sodium hydroxid solution which will be required to neutralize 100 c.c. of the medium. The standard reaction is +1.5, which means that the medium must be of such degree of acidity that 1.5 c.c. of normal solution would be required to neutralize 100 c.c. This corresponds to faint alkalinity to litmus. Most pathogenic bacteria grow better with a reaction of +1.0 or +0.8. Example: If the 10 c.c. which were titrated required 2 c.c. of decinormal solution to bring the pink color, the reaction is +2; and 0.5 c.c. of normal sodium hydroxid must be added to each 100 c.c. of the medium to reduce it to the standard, +1.5.

Tubing Culture-media.—The finished product is stored in flasks or distributed into test-tubes. This is done by means of a funnel fitted with a section of rubber tubing with a glass tip and a pinch-cock. Great care must be exercised, particularly with media which solidify, not to smear any of it upon the inside of the mouth of

the tube, otherwise the cotton stopper will stick. Tubes are generally filled to a depth of 3 or 4 cm. For stab-cultures a greater depth is desired.

After tubing, all culture-media must be sterilized as already described. Agar-tubes are cooled in a slanting position to secure the proper surface for inoculation.

Media should be stored in a cool place, preferably an ice-chest. Evaporation may be prevented by covering the tops of the tubes with tin-foil or the rubber caps which are sold for the purpose; or the cotton stopper may be pushed in a short distance and a cork inserted.

V. STAINING METHODS

In general, bacteria are stained to determine their morphology, their reaction with special methods (*e. g.*, Gram's method), and the presence or absence of certain structures, as spores, flagella, and capsules. Staining methods for various purposes and the formulæ of the staining fluids have been given in previous chapters and can be found by consulting the index. The following will probably be most frequently used:

Methods for tubercle bacilli, pp. 49, 51, and 168.
Methods for capsules of bacteria, pp. 55 and 368.
Methods for *Treponema pallidum*, p. 390.
Löffler's alkaline methylene-blue, p. 57.
Blood-stains, pp. 221–224.

The method of staining bacteria for morphology is as follows, using any simple bacterial stain:

(1) Make a thin smear upon a slide or cover-glass.
(2) Dry in the air, or by warming high above the flame.

(3) " Fix " by passing the preparation, film side up, rather slowly through the flame of a Bunsen burner: a cover-glass three times, a slide about twelve times. Take care not to scorch.

(4) Apply the stain for the necessary length of time, generally one-quarter to one minute.

(5) Wash in water.

(6) Dry by waving high above a flame or by blotting with filter-paper.

(7) Mount by pressing the cover, film side down, upon a drop of Canada balsam on a slide. Slides may be examined with the oil-immersion lens without a cover-glass.

Simple Bacterial Stains.—A simple solution of any basic anilin dye (methylene-blue, basic fuchsin, gentian violet, etc.) will stain nearly all bacteria. These simple solutions are not much used in the clinical laboratory, because other stains, such as Löffler's methylene-blue and Pappenheim's pyronin-methyl-green stain, which serve the purpose even better, are at hand.

Pappenheim's Pyronin-methyl-green Stain.—This solution colors bacteria red and nuclei of cells blue. It is, therefore, especially useful for intracellular bacteria like the gonococcus and the influenza bacillus. It is a good stain for routine purposes, and is a most excellent contrast stain for Gram's method. It colors the cytoplasm of lymphocytes bright red, and has been used as a differential stain for these cells. The solution is applied cold for one-half to five minutes. It consists of saturated aqueous solution methyl-green, 3 to 4 parts, and saturated aqueous solution pyronin, 1 to 1½ parts.

Carbol Thionin.—Saturated solution thionin in 50 per

cent. alcohol, 20 c.c.; 2 per cent. aqueous solution phenol, 100 c.c.

This is especially useful in counting bacteria for a vaccine (p. 422). It can be used as a general stain. In blood work it is used for the malarial parasite and for demonstration of basophilic degeneration of the red cells. It should be preceded by fixation for about a minute in saturated aqueous solution of mercuric chlorid.

Gram's Method.—This is a very useful aid in differentiating certain bacteria and should be frequently resorted to. It depends upon the fact that when treated successively with gentian-violet and iodin, certain bacteria (owing to formation of insoluble compounds) retain the stain when treated with alcohol, whereas others quickly lose it. The former are called *Gram-positive*, the latter, *Gram-negative*. In order to render Gram-negative organisms visible, some contrasting counterstain is commonly applied, but this is not a part of Gram's method proper.

(1) Make smears, dry, and fix by heat.

(2) Apply anilin-gentian-violet or formalin-gentian-violet (p. 57) two to five minutes.

(3) Wash with water.

(4) Apply Gram's iodin solution one-half to two minutes.

(5) Wash in alcohol until the purple color ceases to come off. This is conveniently done in a watch-glass. The preparation is placed in the alcohol, face downward, and one edge is raised and lowered with a needle. As long as any color is coming off, purple streaks will be seen diffusing into the alcohol where the surface of the fluid meets the smear. If forceps be used, beware of stain which may have dried

upon them. The thinner portions of smears from pus should be practically colorless at this stage.

(6) Apply a contrast stain for one-half to one minute. The stains commonly used for this purpose are an aqueous or alcoholic solution of Bismarck brown and a weak solution of fuchsin. In the writer's experience, Pappenheim's pyronin-methyl-green mixture is much more satisfactory; it brings out Gram-negative bacteria more sharply, and is especially desirable for intracellular Gram-negative organisms like the gonococcus and influenza bacillus, since the bacteria are bright red and nuclei of cells blue.

(7) Wash in water, dry, and mount in balsam.

The more important bacteria react to this staining method as follows:

Gram Staining (Deep purple).	Gram Decolorizing (Colorless unless a counterstain is used).
Staphylococcus.	Gonococcus.
Streptococcus.	Meningococcus.
Pneumococcus.	Micrococcus catarrhalis.
Bacillus diphtheriæ.	Bacillus of influenza.
Bacillus tuberculosis.	Typhoid bacillus.
Bacillus of anthrax.	Bacillus coli communis.
Bacillus of tetanus.	Spirillum of Asiatic cholera.
Bacillus aërogenes capsulatus.	Bacillus pyocyaneus.
	Bacillus of Friedländer.
	Koch-Weeks bacillus.
	Bacillus of Morax-Axenfeld.

Moeller's Method for Spores.—Bodies of bacteria are blue, spores are red.

(1) Make thin smears, dry, and fix.

(2) Wash in chloroform for two minutes.

(3) Wash in water.

(4) Apply 5 per cent. solution of chromic acid one-half to two minutes.

(5) Wash in water.

(6) Apply carbol-fuchsin and heat to boiling.

(7) Decolorize in 5 per cent. solution of sulphuric acid.

(8) Wash in water.

(9) Apply 1 per cent. aqueous solution of methylene-blue one-half minute.

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

Löffler's Method for Flagella.—The methods for flagella are applicable only to cultures. Enough of the growth from an agar-culture (which should not be more than eighteen to twenty-four hours old) to produce faint cloudiness is added to distilled water. A small drop of this is placed on a cover-glass, spread by tilting, and dried quickly. The covers must be absolutely free from grease. To insure this, they may be warmed in concentrated sulphuric acid, washed in water, and kept in a mixture of alcohol and strong ammonia. When used they are dried upon a fat-free cloth.

(1) Fix by heating the cover over a flame while holding in the fingers.

(2) Cover with freshly filtered mordant and gently warm for about a minute.

The mordant consists of:

20 per cent. aqueous solution of tannic acid	10 c.c.
Saturated solution ferrous sulphate, cold	5 c.c.
Saturated aqueous or alcoholic solution gentian violet	1 c.c.

(3) Wash in water.

(4) Apply freshly filtered anilin-gentian-violet, warming gently for one-half to one minute.

(5) Wash in water, dry, and mount in balsam.

VI. METHODS OF STUDYING BACTERIA

The purpose of bacteriologic examinations is to determine whether bacteria are present or not, and, if present, their species and comparative numbers. In general, this is accomplished by: 1, Direct microscopic examination; 2, Cultural methods; 3, Animal inoculation.

1. Microscopic Examination.—Every bacteriologic examination should begin with a microscopic study of smears from the pathologic material, stained with a general stain, by Gram's method, and often by the method for the tubercle bacillus. This yields a great deal of information to the experienced worker, and in many cases is all that is necessary for the purpose in view. It will at least give a general idea of what is to be expected, and may determine future procedure.

2. Cultural Methods.—(1) **Collection of Material.**—Material for examination must be collected under absolutely aseptic conditions. It may be obtained with a platinum wire—which has been heated to redness just previously and allowed to cool—or with a swab of sterile cotton on a stiff wire or wooden applicator. Such swabs may be placed in cotton-stoppered test-bubes, sterilized, and kept on hand ready for use. Fluids which contain very few bacteria, and hence require that a considerable quantity be used, may be collected in a sterile hypodermic syringe or one of the pipets described on p. 398. The method of obtaining blood for cultures is given on p. 245.

(2) **Inoculating Media.**—The material is thoroughly distributed over the surface of some solid medium, solid-

ified blood-serum being probably the best for routine work. When previous examination of smears has shown that many bacteria are to be expected, a second tube should be inoculated from the first, and a third from the second, so as to obtain isolated colonies in at least one of the tubes. The platinum wire must be heated to redness before and after each inoculation. When only a few organisms of a single species are expected, as is the case in blood-cultures, a considerable quantity of the material is mixed with a fluid medium.

(3) **Incubation.**—Cultures are placed in an incubator which maintains a uniform temperature, usually of 37.5° C., for eighteen to twenty-four hours, and the growth, if any, is studied as described later. Gelatin will melt with this degree of heat, and must be incubated at about room-temperature.

(4) **Study of Cultures.**—When the original culture contains more than one species, they must be separated, or obtained in "pure culture," before they can be studied satisfactorily. To accomplish this it is necessary to so distribute them on solid media that they form separate colonies, and to inoculate fresh tubes from the individual colonies. In routine work the organisms can be sufficiently distributed by drawing the infected wire over the surface of the medium in a series of streaks. If a sufficient number of streaks be made, some of them are sure to show isolated colonies. Another method of obtaining isolated colonies is to inoculate the water of condensation of a series of tubes, the first from the second, the second from the third, etc., and, by tilting, to flow the water once over the surface of the medium.

In order to determine the species to which an organism belongs it is necessary to consider some or all of the following points:

(1) Naked-eye and microscopic appearance of the colonies on various media.

(2) Comparative luxuriance of growth upon various media. The influenza bacillus, for example, can be grown upon media containing hemoglobin, but not on the ordinary media.

(3) Morphology, special staining reactions, and the presence or absence of spores, flagella, and capsules. Staining methods for these purposes have been given.

(4) Motility. This is determined by observing the living organism with an oil-immersion lens in a hanging-drop preparation, made as follows: A small drop of a bouillon culture or of water of condensation from an agar or blood-serum tube is placed upon the center of a cover-glass; this is inverted over the concavity of a "hollow slide," and ringed with vaselin. In focusing, the edge of the drop should be brought into the field. Great care must be exercised not to break the cover by pushing the objective against it. A method which in some respects is preferable to the hanging drop is to make a ring of vaselin on a slide, place a drop of the culture in this, and apply a cover.

It is not always easy to determine whether an organism is or is not motile, since the motion of currents and the Brownian motion which affects all particles in suspension are sometimes very deceptive.

(5) Production of chemic changes in the media. Among these are coagulation of milk; production of acid in milk and various sugar media to which litmus has been

added to detect the change; production of gas in sugar media, the bacteria being grown in fermentation tubes similar to those used for sugar tests in urine; and production of indol.

(6) Ability to grow without oxygen. For anaërobic methods, the reader is referred to larger works.

(7) Effects produced when inoculated into animals.

3. Animal Inoculation.—In clinical work this is resorted to chiefly to detect the tubercle bacillus. The method is described on p. 375.

For the study of bacteria in cultures, a small amount of a pure culture is injected subcutaneously or into the peritoneal cavity. The animals most used are guinea-pigs, rabbits, and mice. For intravenous injection the rabbit is used because of the easily accessible marginal vein of the ear.

VII. CHARACTERISTICS OF SPECIAL BACTERIA

Owing to the great number of bacterial species, most of which have not been adequately studied, positive identification of an unknown organism is often a very difficult problem. Fortunately, however, only a few are commonly encountered in routine work, and identification of these with comparative certainty presents no great difficulty. Their more distinctive characteristics are outlined in this section.

1. **Staphylococcus Pyogenes Aureus.**—The morphology and staining reactions (described on p. 368) and the appearance of the colonies are sufficient for diagnosis. Colonies on solidified blood-serum and agar are rounded, slightly elevated, smooth and shining, and vary in color

from light yellow to deep orange. Young colonies are sometimes white.

2. **Staphylococcus Pyogenes Albus.**—This is similar to the above, but colonies are white. It is generally less virulent.

3. **Staphylococcus Pyogenes Citreus.**—The colonies are lemon yellow; otherwise it resembles the white staphylococcus.

4. **Streptococcus Pyogenes.**—The morphology and staining reactions have been described (p. 368). The chains are best seen in the water of condensation and in bouillon cultures. The cocci are not motile. Colonies on blood-serum are minute, round, grayish, and translucent. Litmus milk is usually acidified and coagulated, although slowly. The streptococcus rarely produces acid in Hiss' serum-water-litmus-inulin medium (see p. 405).

5. **Pneumococcus.**—The only organism with which this is likely to be confused is the streptococcus. The distinction is often extremely difficult.

Detection of the pneumococcus in fresh material has been described (p. 54). In cultures it frequently forms long chains. Capsules are not present in cultures except upon special media. They show best upon a serum medium like that described for the gonococcus, but can frequently be seen in milk. Colonies on blood-serum resemble those of the streptococcus. The pneumococcus usually promptly acidifies and coagulates milk, and acidifies and coagulates Hiss' serum-water with inulin. The latter property is very helpful in diagnosis.

6. **Gonococcus.**—Its morphology and staining pecu-

liarities are given on p. 369. These usually suffice for its identification, cultural methods being rarely undertaken. In cultures the chief diagnostic point is its failure to grow on ordinary media. To grow it the most convenient medium is made by adding ascitic or hydrocele fluid (which has been obtained under aseptic conditions) to melted agar in proportion of 1 part of serum to 3 parts of agar. The agar in tubes is melted and cooled to about 45° C.; the serum is added with a pipet and mixed by shaking; and the tubes are again cooled in a slanting position. Colonies upon this medium are minute, grayish, and translucent.

7. **Diplococcus Intracellularis Meningitidis.**—It grows poorly or not at all on plain agar. On Löffler's blood-serum, upon which it grows fairly well, colonies are round, colorless or hazy, flat, shining, and viscid looking. It quickly dies out.

8. **Diphtheria Bacillus.**—The diagnosis is usually made from a study of stained smears from cultures upon blood-serum. Its morphology and staining peculiarities are characteristic when grown on this medium (see p. 379). The bacilli are non-motile and Gram-positive. The colonies are round, elevated, smooth, and grayish.

9. **Typhoid and Colon Bacilli.**—These are medium-sized, motile, Gram-negative, non-spore-bearing bacilli. Upon blood-serum they form rounded, grayish, slightly elevated, viscid looking colonies, those of the colon bacillus being somewhat the larger. They do not liquefy gelatin. They represent the extremes of a large group with many intermediate types. They are distinguished as follows:

Typhoid Bacillus.	*Colon Bacillus.*
Actively motile.	Much less active.
Growth on potato usually invisible.	Growth distinctly visible as dirty gray or brownish slimy layer.
No gas produced in glucose media.	Produces gas.
Growth in litmus milk produces no change.	Litmus milk pink and coagulated.
Produces no indol in Dunham's peptone medium.	Produces indol. (For test, see p. 404.)
Agglutinates with serum from typhoid-fever patient. (Recently isolated bacilli do not agglutinate well.)	Does not agglutinate with typhoid serum.

10. **Bacillus of Influenza.**—Diagnosis will usually rest upon the morphology and staining peculiarities, described on p. 58, and upon the fact that the bacillus will not grow on ordinary media, but does grow upon hemoglobin-containing media. It can be grown upon agar-slants which have been smeared with a drop of blood from a puncture in the finger. Before inoculation these slants should be incubated to make sure of sterility. The colonies are difficult to see without a hand lens. They are very minute, discrete, and transparent, resembling small drops of dew.

11. **Bacillus of Tuberculosis.**—The methods of identifying this important organism have been given (pp. 49 and 168). Cultivation is not resorted to in clinical work. It grows very slowly and only on certain media. It is Gram-positive and non-motile.

CHAPTER IX

PREPARATION AND USE OF VACCINES

BACTERIAL vaccines, sometimes called "bacterins," which within the past few years have come to play an important rôle in therapeutics, are suspensions of definite numbers of dead bacteria in normal salt solution. While in many cases, notably in gonorrhea and tuberculosis, ready prepared or "stock" vaccines are satisfactory, it is usually desirable and often imperative for best results to use vaccines which are especially prepared for each patient from bacteria which have been freshly isolated from his own lesion. These latter are called "autogenous vaccines." Only through them can one be certain of getting the exact strain of bacterium which is producing the disease.

I. PREPARATION OF VACCINE

The method of preparing autogenous vaccines which is used in the author's laboratory is here described.

1. Preparation of Materials.—A number of 2-ounce wide-mouthed bottles are cleaned and sterilized. Each is charged with 50 c.c. freshly filtered normal salt solution (0.85 per cent. sodium chlorid in distilled water), and is capped with a new rubber nursing-nipple, without holes, inverted as shown in Fig. 162. A small section of hollow wire or a hypodermic needle is thrust through the cap near the edge to serve as an air vent, and the bottle and

contents are sterilized in an autoclave. If an autoclave is not at hand, successive steamings in an Arnold sterilizer will answer, provided it is not opened between steamings. After sterilization, the pieces of wire are pulled out and the holes sealed with collodion.

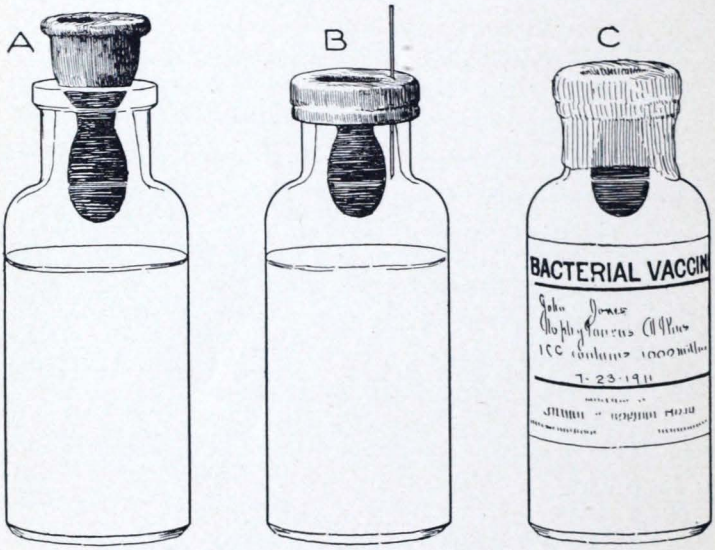

Fig. 162.—Vaccine bottles: A, Cap ready to be applied; B, ready for sterilization; C, finished vaccine.

A number of test-tubes, each charged with 10 c.c. of normal salt solution and plugged with cotton, are also prepared and sterilized.

2. Obtaining the Bacteria.—A culture on some solid medium is made from the patient's lesion, and a pure culture is obtained in the usual way. This preliminary work should be carried through as quickly as possible. If for any reason there is much delay, it is best to begin over again, the experience gained in the first trial en-

abling one to carry the second through more rapidly. When a pure culture is obtained, a number of tubes of blood-serum or agar—10 or 12 in the case of streptococcus or pneumococcus, 4 or 5 in the case of most other organisms—are planted and incubated over night or until a good growth is obtained.

3. Making the Suspension.—The salt solution from one of the 10 c.c. salt-tubes is transferred by means of a

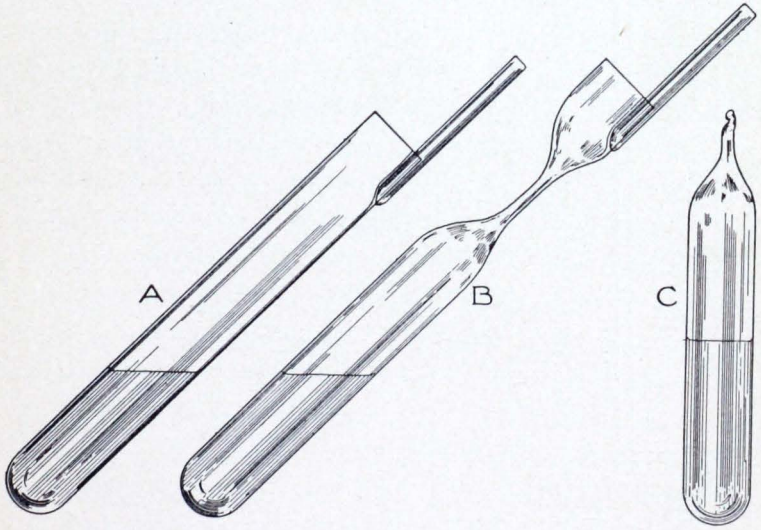

Fig. 163.—Process of making hermetically sealed capsules.

sterile pipet to the culture-tubes, and the growth thoroughly rubbed up with a stiff platinum wire or a glass rod whose tip is bent at right angles. The suspension from the different tubes, usually amounting to about 10 c.c., is then collected in one large tube (size about 6 by $\frac{3}{4}$ inch); and the upper part of the tube is drawn out in the flame of a blast lamp or Bunsen burner, as indicated in Fig. 163, a short section of glass tubing being fused to

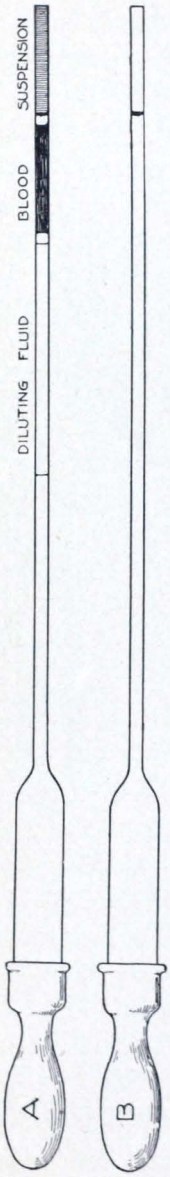

Fig. 164.—Pipets used in counting vaccines by Wright's method. The slender portion should be narrower than here represented.

the rim of the tube to serve as a handle. It is then stood aside, and when cool the end is sealed off.

The resulting hermetically sealed capsule is next thoroughly shaken for ten to twenty minutes to break up all clumps of bacteria. Some small pieces of glass or a little clean sterile sand may be introduced to assist in this, but with many organisms it is not necessary.

4. Sterilization.—The capsule is placed in a water-bath at 60° C. for forty-five minutes. This can be done in an ordinary rice-cooker, with double lid through which a thermometer is inserted. When both compartments are filled with water it is an easy matter to maintain a uniform temperature by occasional application of a small flame. The time and temperature are important: too little heat will fail to kill the bacteria, and too much will destroy the efficiency of the vaccine.

When sterilization is complete the capsule is opened, a few drops are planted on agar or blood-serum, and the capsule is again sealed.

5. Counting.—When incubation of the plant has shown the suspension to be sterile it is ready for counting.

There must be ready a number of clean slides; a few drops of normal salt solution on a slide or in a watch-glass; a blood-lancet, which can be improvised from a spicule of glass or a pen; and two slender pipets with squarely broken off tips and grease-pencil marks about 2 cm. from the tip (Fig. 164). These are easily made by drawing out a piece of glass tubing, as described on page 398.

It is necessary to work quickly. After thorough shaking, the capsule is opened and a few drops forced out upon a slide. Any remaining clumps of bacteria are broken up with one of the pipets by holding it against and at right angles to the slide, and alternately sucking the fluid in and forcing it out. The pipet is most easily controlled if held in the whole hand with the rubber bulb between the thumb and the side of the index-finger. A finger is then pricked until a drop of blood appears; and into the second pipet are quickly drawn successively: 1 or 2 volumes normal salt solution (or better, a 1 per cent. solution of sodium citrate which prevents coagulation); a small bubble of air; 1 volume of blood; a small bubble of air; and, finally, 1 volume of bacterial suspension. (A "volume" is measured by the distance from the tip of the pipet to the grease-pencil mark.) The contents of the pipet are then forced out upon a slide and thoroughly mixed by sucking in and out, care being taken to avoid bubbles; after which the fluid is distributed to a number of slides and spread as in making blood-smears.

The films are stained with Wright's blood-stain or, better, by a few minutes' application of carbol-thionin, after fixing for a minute in saturated mercuric chlorid solution. With an oil-immersion lens both the red cells

and the bacteria in a number of microscopic fields are counted. The exact number is not important; for convenience 500 red cells may be counted. From the ratio between the number of bacteria and of red cells, it is easy to calculate the number of bacteria in 1 c.c. of the suspension, it being known that there are 5000 million red corpuscles in a cubic centimeter of normal human blood. If there were twice as many bacteria as red corpuscles in the fields counted, the suspension would contain 10,000 million bacteria per cubic centimeter.

The count can also be made with the hemocytometer, using a weak carbol-fuchsin or gentian violet, *freshly filtered*, as diluting fluid. A very thin cover-glass must be used; and, after filling, the counting-chamber must be set aside for an hour or more to allow the bacteria to settle. Mallory and Wright advise the use of the shallow chamber manufactured by Zeiss for counting blood-plates, but many 2 mm. oil-immersion objectives have sufficient working distance to allow the use of the regular Thoma counting-chamber, provided a very thin cover is used.

6. Diluting.—The amount of the suspension, which, when diluted to 50 c.c., will give the strength desired for the finished vaccine having been determined, this amount of salt solution is withdrawn with a hypodermic syringe from one of the bottles already prepared, and is replaced with an equal amount of suspension. One-tenth c.c. of trikresol or lysol is finally added and the vaccine is ready for use. To prevent possible leakage about the cap, the neck of the bottle is dipped in melted paraffin. The usual strengths are: staphylococcus, 1000 million in 1 c.c.; most other bacteria, 100 million in 1 c.c.

II. METHOD OF USE

Vaccines are administered subcutaneously, usually in the arm or abdominal wall or between the shoulder-blades. The rubber cap is sterilized by filling the concavity with alcohol for some minutes, usually while the syringe is being sterilized. The bottle is then inverted and well shaken, when the needle is plunged through the rubber and the desired quantity withdrawn. The hole seals itself and no collodion is necessary, which is one of the advantages of this form of cap. The most satisfactory syringe is the comparatively inexpensive "Sub-Q Tuberculin" syringe graduated in hundredths of a cubic centimeter.

A rapid and efficient technic for giving the injections is suggested by Major F. T. Woodbury of the Army Medical Corps. The needle is dipped into tincture of iodin and some is drawn into the syringe and expelled; a small quantity of the vaccine or of sterile water is likewise drawn in and expelled, after which the dose to be given is drawn in. The patient's arm is touched with a swab of cotton saturated with tincture of iodin and the injection is made through the resulting brown spot. The syringe is cleaned by drawing into it and expelling, successively, tincture of iodin, alcohol, and air.

III. DOSAGE

Owing to variations, both in virulence of organisms and susceptibility of patients, no definite dosage can be assigned. Each case is a separate problem. Wright's original proposal was to regulate the size and frequency of dose by its effect upon the opsonic index, but this is

beyond the reach of the practitioner. The more widely used "clinical method" consists in beginning with a very small dose and cautiously increasing until the patient shows either improvement or some sign of a "reaction," indicated by headache, malaise, fever, exacerbation of local disease, or inflammatory reaction at the site of injection. The reaction indicates that the dose has been too large. The beginning dose of staphylococcus is about 50 million; the maximum, 1000 million or more. Of most other organisms the beginning dose is 5 million to 10 million; maximum, about 100 million, Ordinarily, injections are given once or twice a week; very small doses may be given every other day.

IV. THERAPEUTIC INDICATIONS

The therapeutic effect of vaccines depends upon their power to stimulate the production of opsonins and other antibacterial substances which enable the body to combat the infecting bacteria. Their especial field is the treatment of subacute and chronic localized infections, in some of which they offer the most effective means of treatment at our command. In most chronic infections the circulation of blood and lymph through the diseased area is very sluggish, so that the antibodies, when formed, cannot readily reach the seat of disease. Ordinary measures which favor circulation in the diseased part should, therefore, accompany the vaccine treatment. Among these may be mentioned incisions and drainage of abscesses, dry cupping, application of heat, Bier's hyperemia, etc., but such measures should not be applied during the twenty-four hours succeeding an injection, since the first effect of the vaccine may be a temporary

lowering of resistance. Vaccines are of little value, and, in general, are contraindicated in very acute infections, particularly in those which are accompanied by much systemic intoxication, for in such cases the power of the tissues to produce antibodies is already taxed to the limit. It is true, nevertheless, that remarkably beneficial results have occasionally followed their use in such acute conditions as malignant endocarditis, but here they should be tried with extreme caution.

Probably best results are obtained in staphylococcus infections, although pneumococcus, streptococcus, and colon bacillus infections usually respond nicely. Among clinical conditions which have been treated successfully with vaccines are furunculosis, acne vulgaris, infected operation-wounds, pyelitis, cystitis, subacute otitis media, osteomyelitis, infections of nasal accessory sinuses, etc. Vaccine treatment of the mixed infection is doubtless an important aid in tuberculosis therapy, and in some cases the result is brilliant. When, as is common, several organisms are present in the sputum, a vaccine is made from each, excepting the tubercle bacillus, of which autogeneous vaccines are not used in practice. To avoid the delay and consequent loss of virulence entailed by study and isolation of the several varieties, many workers make the bacterial suspension directly from the primary cultures. The resulting vaccines contain all strains which are present in the sputum in approximately the same relative numbers. Although open to criticism from a scientific standpoint, this method offers decided practical advantages in many cases.

It has been shown that vaccines are useful in prevent-

ing as well as curing infections. Their value has been especially demonstrated in typhoid fever. Three or four doses of about 50,000,000 typhoid bacilli are given about seven days apart. Results in the army, where the plan has been tried on a large scale, show that such vaccination is effective.

V. TUBERCULINS

Tuberculins contain the products of tubercle bacilli or their ground-up bodies, the latter class being practically vaccines. They are undoubtedly of great value in the treatment of localized tuberculosis, particularly of bones, joints, and glands; and are of rather indefinite though certainly real value in chronic pulmonary tuberculosis, especially when quiescent. The best known are Koch's old tuberculin (T. O.), bouillon filtrate (B. F.), triturate residue (T. R.), and bacillary emulsion (B. E.). There seems to be little difference in the actions of these, although theoretically T. R. should immunize against the bacillus and B. F. against its toxic products. The choice of tuberculin is much less important than the method of administration. The making of autogenous tuberculins is impracticable, hence stock preparations are used in practice.

Since the dose is exceedingly minute, the tuberculin as purchased must be greatly diluted before it is available for use. A convenient plan is to use the rubber-capped 50 c.c. bottles of sterile salt solution described for vaccines (p. 419), adding sufficient tuberculin to give the desired strength, together with 0.1 c.c. trikresol to insure sterility. The practitioner should bear in mind that while tuberculin is capable of good, it is also capable

of great harm. Everything depends upon the dosage and plan of treatment. Probably a safe beginning dose for a pulmonary case is 0.00001 milligram of B. E., B. F., or T. R.; for gland and bone cases, about 0.0001 milligram. O. T. is now used chiefly in diagnosis. The intervals are about one week or, rarely, three days, when very small doses are given. The dose is increased steadily, but with *extreme caution;* and should be diminished or temporarily omitted at the first indication of a "reaction," of which, in general, there are three forms:

(*a*) *General:* Elevation of temperature (often slight), headache, malaise.

(*b*) *Local:* Increase of local symptoms, amount of sputum, etc.

(*c*) *Stick:* Inflammatory reaction at site of injection.

Treatment is usually continued until a maximum dose of 1 mm. is reached, the course extending over a year or more.

VI. TUBERCULIN IN DIAGNOSIS

The tissues of a tuberculous person are sensitized toward tuberculin, and a reaction (see preceding section) occurs when any but the most minute quantity of tuberculin is introduced into the body. Non-tuberculous persons exhibit no such reaction. This is utilized in the diagnosis of obscure forms of tuberculosis, the test being applied in a number of ways:

1. **Hypodermic Injection.**—Koch's old tuberculin is used in successive doses, several days apart, of 0.001, 0.01, and 0.1 mg. A negative result with the largest amount is considered final. The reaction is manifested by fever within eight to twenty hours after the injection. The method involves some danger of lighting up a

latent process, and has been largely displaced by safer methods.

2. **Calmette's Ophthalmo-reaction.**—One or two drops of 0.5 per cent. purified old tuberculin are instilled into one eye. Tuberculin ready prepared for this purpose is on the market. If tuberculosis exists anywhere in the body, a conjunctivitis is induced within twelve to twenty-four hours. This generally subsides within a few days. The method is not without some, though slight, risk of injury to the eye; and the test is absolutely contraindicated in the presence of any form of ocular disease. A second instillation should not be tried in the same eye.

3. **Moro Reaction.**—A 50 per cent. ointment of old tuberculin in lanolin is rubbed into the skin of the abdomen, a piece about the size of a pea being required. Dermatitis, which appears in twenty-four to forty-eight hours, indicates a positive reaction. The ointment can be purchased ready for use.

4. **Von Pirquet's Method.**—This is the most satisfactory of the tuberculin tests. Two small drops of old tuberculin are placed on the skin of the front of the forearm, about 2 inches apart, and the skin is slightly scarified, first at a point midway between them, and then through each of the drops. A convenient scarifier is a piece of heavy platinum wire, the end of which is hammered to a chisel edge. This is held at right angles to the skin, and rotated six to twelve times with just sufficient pressure to remove the epidermis without drawing blood. In about ten minutes the excess of tuberculin is gently wiped away with cotton. No bandage is necessary. A positive reaction is shown by the appearance in

twenty-four to forty-eight hours of a papule with red areola, which contrasts markedly with the small red spot left by the control scarification.

These tests have very great diagnostic value in children, especially those under three years of age, but are often misleading in adults, positive reactions occurring in many apparently healthy individuals. Negative tests are very helpful in deciding against the existence of tuberculosis.

APPENDIX

I. APPARATUS, REAGENTS, AND STAINS

THE apparatus and reagents listed here are sufficient for all but the rarer tests described in the text. Those in smaller type are less frequently required. For ordinary routine work a much smaller list will suffice.

A. APPARATUS

Beakers and flasks, several sizes, preferably of Jena glass.

Blood lancet, or some substitute (Fig. 68).

Bunsen-burner or alcohol lamp.

Buret, 25 c.c. capacity, preferably with Schellbach stripe.

Buret and filter-stand combined.

Centrifuge—hand, electric, or water-power (Figs. 20 and 21). With the last two a speed indicator is desirable. Radius of arm when in motion should be $6\frac{3}{4}$ inches. Plain and graduated tubes accompany the instrument; milk-tubes (Fig. 157) must be purchased separately. The hematocrit attachment (Fig. 77) is not much used.

Cigaret-paper, "Zig-zag" brand or some similar thin paper, for making blood-films.

Corks, preferably of rubber, with one and two holes.

Cover-glasses, No. 2 thickness—$\frac{7}{8}$-inch squares are most convenient.

Cover-glass forceps.

Esbach's tube (Fig. 27).

Evaporating dish.

Filter-paper: ordinary cheap paper for urine filtration; "ashless" quantitative filter-paper for chemic analyses.

Glass funnels.

Glass rods and tubing of sodium glass: for stirring rods, urinary pipets, etc.

Glass slides: the standard 1- by 3-inch size will answer for all work, although a few larger slides will be found convenient; those of medium thickness are preferable.

Graduates, cylindric form, several sizes.

Graniteware basin.

Hemoglobinometer: see pp. 185 to 191 for descriptions of the different instruments.

Hemocytometer: either Türck or Zappert ruling is desirable (Figs. 73, 74, and 79).

Hypodermic syringe: the "Aseptic Sub-Q, Tuberculin," is probably the most useful type.

Incubator (p. 397).

Labels for slides and bottles.

Litmus-paper, red and blue, Squibb's preferred.

Mett's tubes (pp. 300 and 399).

Microscope (Fig. 1). Equipment described on p. 31.

Petri dishes.

Platinum wires (p. 397).

Sterilizers: the Arnold type for steaming; oven for dry sterilization (p. 396).

Stomach-tube.

Test-glass, conic, one side painted half white, half black.

Test-tubes, rack, and cleaning brush.

Ureometer, Doremus-Hinds' pattern (Fig. 24).

Urinometer, preferably Squibb's (Fig. 17).

Blood-fixing oven, or Kowarsky's plate (Fig. 84).

Copper-foil and gauze.

Cotton, absorbent, for filtering, etc.

" Cotton-batting " for plugging tubes.

Culture-media. The selection depends upon the work to be done (p. 401).

Holt's cream gage and hydrometer (Fig. 156).

Horismascope (Fig. 26).

Pipets, graduated, 5 to 50 c.c. capacity.

Ruhemann's tube for uric-acid estimation (Fig. 25).

Saccharimeter (Fig. 29).

Strauss' separatory funnel for lactic-acid test (Fig. 105).

Suction filter.

Urinopyknometer of Saxe (Fig. 18).

Widal reaction outfit: either living agar cultures of the typhoid bacillus, or the dead cultures with diluting apparatus, which are sold under various trade names.

Water-bath.

B. REAGENTS AND STAINS

All stains and many reagents are best kept in small dropping bottles. Formulæ are given in the text. Dry stains (Grübler's should be specified) and most staining solutions and chemical reagents can be purchased of Bausch & Lomb Optical Co., Rochester, New York, or Eimer & Amend, New York. For the physician who does only a small amount of work, the " Soloid " tablets manufactured by Burroughs, Wellcome & Co. are convenient and satisfactory. These tablets have only to be dissolved in a specified amount of fluid to produce the finished stain. Most of the stains mentioned here come in this form.

Acid, glacial acetic. Other strengths can be made from this as desired.

Acid, hydrochloric, concentrated (contains about 32 per cent. by weight of absolute hydrochloric acid). Other strengths can be made as desired.

Acid, nitric, strong, colorless.

Acid, nitric, yellow. Can be made from colorless acid by adding a splinter of pine, or allowing to stand in sunlight.

Acid, sulphuric, concentrated.

Alcohol, ethyl (grain-alcohol). This is ordinarily about 93 to 95 per cent., and other strengths can be made as desired.

Aqua ammoniæ fortior (sp. gr. 0.9).

Bromin, or Rice's solutions (p. 95), for urea estimation.

Chloroform.

Diluting fluid for erythrocyte count (p. 198).

Diluting fluid for leukocyte count (p. 213).

Dimethyl-amido-azobenzol, 0.5 per cent. alcoholic solution.

Distilled water.

Esbach's or Tsuchiya's reagent (p. 105).

Ether, sulphuric.

Ferric chlorid: saturated aqueous solution and 10 per cent. aqueous solution.

Haines' (or Fehling's or Benedict's) solution (pp. 109, 110).

Lugol's solution (*Liquor Iodi Compositus*, U. S. P.). Gram's iodin solution (p. 57) can be made from this by adding fourteen times its volume of water.

Obermayer's reagent (p. 91).

Phenylhydrazin, pure.

Phenol.

Phenolphthalein, 1 or 0.5 per cent. alcoholic solution.

Purdy's (or Fehling's or Benedict's) solution (pp. 112–115).

Robert's reagent (p. 103).

Sodium chlorid (table salt), saturated aqueous solution.

Sodium hydroxid (caustic soda), 40 per cent. solution; other strengths can be made from this as desired.

Sodium hydroxid, decinormal solution. The practitioner will find it best to purchase this solution ready prepared. Eimer and Amend, New York, and many other chemical supply houses carry it in stock. For ordinary clinical work 41 grams of Merck's "sodium hydrate by alcohol" from a freshly opened bottle may be dissolved in 1000 c.c. water. This makes a normal solution and must be diluted with 9 volumes of water to make the decinormal solution.

Sodium nitrite, 0.5 per cent. solution for diazo-reaction. Must be freshly prepared.

Sulphanilic acid solution for diazo-reaction (p. 128).

Stains:

Carbol fuchsin (p. 50).

Eosin, saturated aqueous solution.

Formalin-gentian-violet, or anilin-gentian-violet (p. 57).

Gabbet's stain or Pappenheim's methylene-blue stain (p. 51).

Löffler's alkaline methylene-blue solution (p. 57).

Pappenheim's pyronin-methyl-green stain (p. 408).

Stain for fat: Sudan III, saturated solution in 70 per cent. alcohol; or 1 per cent. aqueous solution osmic acid.

Wright's or Harlow's stain for blood.

Tincture of guaiac, diluted to a light sherry-wine color (keep in a dark glass bottle).

Turpentine, "ozonized" (p. 125).

Acid, boric, for preserving urine (p. 69).

Acid, oxalic.

Acid, salicylous (salicyl aldehyd), 10 per cent. alcoholic solution.

Alcohol, amylic.

Alcohol, ethyl, absolute.

Alcohol, methyl (pure).

Antiformin (p. 52).

Barium chlorid mixture (p. 89).

Benzol.

Boas' reagent or Günzburg's (p. 290).

Boggs' reagent (p. 387).

Calcium chlorid, 1 per cent. solution.

Canada-balsam in xylol: necessary only when permanent microscopic preparations are made.

Carbon disulphid.

Charcoal, animal.

Chromium trioxid.

Congo-red, strong alcoholic solution.

Copper sulphate.

Diluting fluid for blood-platelet count (pp. 215, 216).

Egg-albumen discs in glycerin (p. 293).

Ether, acetic, pure.

Florence's reagent (p. 393).

Formalin (40 per cent. solution of formaldehyd gas).

India-ink (Günther and Wagner) (p. 391).

Iodin crystals.

Iron sulphid.

Lead acetate (sugar of lead); used in 10 per cent. solution to clarify urine.

Lead acetate, tribasic.

Lime-water.

Müller's fluid saturated with mercuric chlorid (p. 56).

Orcin.

Pepsin, U. S. P.

Phenylhydrazin hydrochlorid.

Potassium ferrocyanid, 10 per cent. solution.

Potassium oxalate (neutral).

Potassium persulphate.

Ruhemann's reagent (p. 97).

Silver nitrate crystals; also dram to the ounce aqueous solution, and " ammoniated " solution (p. 97).

Sodium alizarin sulphonate, 1 per cent. aqueous solution.

Sodium carbonate.

Sodium chlorid, 2 per cent. solution; from this normal salt solution (0.8 per cent.) can be made as desired.

Sodium hyposulphite.

Sodium nitroprussid.

Sodium sulphate.

Stains:

Bismarck-brown, saturated aqueous or alcoholic solution.

Carbol-thionin.

Ehrlich's triple stain for blood.

Eosin, 0.5 per cent. alcoholic solution for blood.

Fuchsin, weak solution; can be made when desired by adding a little carbol-fuchsin to a test-tube of water.

Gentian-violet, saturated alcoholic solution.

Giemsa's stain (p. 390).

Methylene-blue and borax solution (p. 254).

Methylene-blue, saturated aqueous solution for blood.

Van Gieson's stain for Negri bodies (p. 395).

Sulphur, powdered.

Talc, purified (Talcum Purificatum, U. S. P.).

Trichloracetic acid solution (p. 102).

Uranium nitrate, 5 per cent. aqueous solution.
Xylol.
Zinc, arsenic free.

II. WEIGHTS, MEASURES, ETC., WITH EQUIVALENTS

METRIC

Meter (unit of length): Millimeter (mm.) = $\frac{1}{1000}$ meter. Centimeter (cm.) = $\frac{1}{100}$ meter. Kilometer = 1000 meters. Micron (μ) = $\frac{1}{1000}$ millimeter.

Gram (unit of weight): Milligram (mg.) = $\frac{1}{1000}$ gram. Kilogram (kilo.) = 1000 meters.

Liter (unit of capacity): Cubic Centimeter = $\frac{1}{1000}$ liter. Same measure as milliliter (ml.).

1 Millimeter = { 0.03937 ($\frac{1}{25}$ approx.) in. ; 1000 microns.
1 Centimeter = { 0.3937 ($\frac{2}{5}$ approx.) in. ; 0.0328 feet.
1 Meter = { 39.37 in. ; 3.28 feet.
1 Micron (μ) = { $\frac{1}{25000}$ in. ; 0.001 millimeter.

1 Gram = { 15.43 grains. ; 0.563 dram ; 0.035 ounce ; 0.0022 pound } Avoir. ; 0.257 dram ; 0.032 ounce ; 0.0027 pound } Apoth.
1 Kilogram = { 35.27 ounce (Avoir.). ; 2.2 pound (Avoir.).
1 Liter = { 1.056 (1 approx.) quart. ; 61.02 cu. inches. ; 1000 cu. centimeters.

1 Sq. Millimeter = 0.00155
1 Sq. Centimeter = 0.1550 } sq. in.
1 Sq. Meter = 1550
1 Sq. Meter = 10.76 sq. feet.

1 Cu. Millimeter = 0.00006
1 Cu. Centimeter = 0.0610 } cu. in.
1 Cu. Centimeter = 0.001 liter.
1 Cu. Meter = { 35.32 cu. feet. ; 61025.4 cu. in.

1 Inch = 25.399 millimeters.
1 Sq. Inch = 6.451 sq. centimeters.
1 Cu. Inch = 16.387 cu. centimeters.

1 Foot = 30.48 centimeters.
1 Sq. Foot = 0.093 sq. meter.
1 Cu. Foot = 0.028 cu. meter.

AVOIRDUPOIS WEIGHT

1 Ounce = { 437.5 grains. ; 16 drams.
1 Pound = 16 ounces.

1 Grain = 0.065 ($\frac{3}{50}$ approx.)
1 Dram = 1.77 ($1\frac{3}{4}$ approx.)
1 Ounce = 28.35 (30 approx.)
1 Pound = 453.59 (500 approx.) } grams.
1 Pound = 27.7 cu. inches.
1 Pound = 1.215 lb. Troy.

APOTHECARIES' MEASURE

1 Dram = 60 minims.
1 Ounce = 8 drams.
1 Pint = 16 ounces.
1 Gallon = 8 pints.

1 Dram = 3.70
1 Ounce = 29.57
1 Pint = 473.1
1 Gallon = 3785.4 } cu. centimeters.
1 Gallon = 231 cu. inches.

APOTHECARIES' WEIGHT

1 Scruple = 20 grains.
1 Dram = 3 scruples = 60 grains.
1 Ounce = 8 drams = 480 grains.
1 Pound = 12 ounces.

1 Grain = 0.065
1 Dram = 3.887
1 Ounce = 31.10
1 Pound = 373.2
} grams.

To convert		*minims*	into	*cubic centimeters*	multiply by		0.061
"	"	*fluidounces*	"	*cubic centimeters*	"	"	29.57
"	"	*grains*	"	*grams*	"	"	0.0648
"	"	*drams*	"	*grams*	"	"	3.887
"	"	*cubic centimeters*	"	*minims*	"	"	16.23
"	"	*cubic centimeters*	"	*fluidounces*	"	"	0.0338
"	"	*grams*	"	*grains*	"	"	15.432
"	"	*grams*	"	*drams*	"	"	0.257

TEMPERATURE

CENTIGRADE.	FAHRENHEIT.	CENTIGRADE.	FAHRENHEIT.
110°	230°	37°	98.6°
100	212	36.5	97.7
95	203	36	96.8
90	194	35.5	95.9
85	185	35	95
80	176	34	93.2
75	167	33	91.4
70	158	32	89.6
65	149	31	87.8
60	140	30	86
55	131	25	77
50	122	20	68
45	113	15	59
44	111.2	10	50
43	109.4	+5	41
42	107.6	0	32
41	105.8	—5	23
40.5	104.9	—10	14
40	104	—15	+5
39.5	103.1	—20	—4
39	102.2		
38.5	101.3	0.54° =	1°
38	100.4	1 =	1.8
37.5	99.5	2 =	3.6
		2.5 =	4.5

To convert Fahrenheit into Centigrade, subtract 32 and multiply by 0.555.

To convert Centigrade into Fahrenheit, multiply by 1.8 and add 32.