solutions The amount required to saturate will vary from two to five per cent.

The concentrated alcoholic solutions are never used as such, but serve for the preparation of the dilute dyes which are the stains proper. The latter are made by

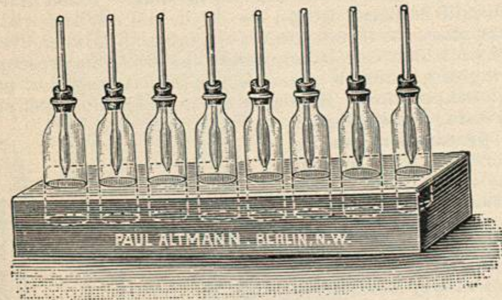

FIG. 5108.—Stand for Staining Solutions.

placing a few cubic centimetres of the concentrated dye in a small tincture bottle, and adding ten to twenty parts of water. This bottle is then provided with a cork and a piece of glass tubing which serves as a pipette. The different dilute dyes can be kept in a stand, such as is shown in Fig. 5108. The dilute dyes after a while undergo alteration and yield deposits. In that event they should be filtered before use, or else new dilutions should be made.

The acid aniline dyes are represented by eosin, acid fuchsin, and fluorescein. The concentrated and the dilute dyes are prepared as above.

The staining solutions may be used as such, or their properties may be accentuated by the addition of substances which act more or less directly as mordants. A number of these solutions are in daily use, and for that reason their preparation is here given.

Löffler's methylene blue is made by adding 30 c.c. of concentrated methylene blue to 100 c.c. of a 0.01-per-cent. solution of potassium hydrate. A similar solution with less alkali was first used by Koch. The alkali not only serves to make the cell more permeable, but also increases the staining power by liberating the free base from the dye.

Carbolic fuchsin, or Ziehl solution, is made by adding 1 gm. of fuchsin and 10 c.c. of alcohol to 100 c.c. of a five-per-cent. carbolic-acid solution. This stain is very widely used for simple as well as double staining. Czaplewski modified it by substituting glycerin for the alcohol. His solution is prepared by rubbing up in a mortar 1 gm. of fuchsin with 5 gm. of carbolic acid, and to this 150 gm. of glycerin and 100 c.c. of water are added.

Carbolic methylene blue, first employed by Kühne, consists of 1.5 gm. of methylene blue, 10 gm. of absolute alcohol, and 100 c.c. of a five-per-cent. solution of carbolic acid.

Carbolic theonin consists of 10 parts of a saturated solution of thionin and 100 parts of a one-per-cent. solution of carbolic acid (Nicolle).

Carbolic gentian violet is made the same as the preceding (Nicolle).

Aniline Water, Gentian Violet, etc.—The carbolic acid, like the alkali, favors the penetration of the stain. Aniline water acts in like manner and was first used by Ehrlich. To prepare the aniline water a few cubic centimetres of aniline are placed in a test tube, and this is then filled with distilled water and thoroughly shaken. The milky liquid is filtered through a moist filter. To the water-clear filtrate enough concentrated fuchsin or gentian violet is then added to make the liquid opaque, and so that it just begins to form on the surface a slight metallic film of precipitated dye. The solution is then used as such, but if the deposit is very marked it may be necessary first to filter it. The aniline water dyes do not keep very well, and for that reason it is well to make a fresh solution every time that it is to be used. Oil of cloves has been suggested by London as a substitute for aniline.

The aniline water stains were first employed by Ehrlich for coloring the tubercle bacillus, and are still used for that purpose. They are, however, employed especially for staining whips and in connection with Gram's stain. In the latter case, after the preparation has been stained with the solution, a mordant is applied, known as Lugol's solution, which serves to form a difficultly soluble compound between the dye and the cell contents.

Lugol's solution consists of 1 part of iodine, 2 parts of potassium iodide, and 300 parts of distilled water.

The Staining of Cover-glass Preparations.—These may be considered under the head of (1) simple, (2) double, and (3) special stains. For the simple stains, when it is desired to have a heavily colored preparation, either fuchsin or gentian violet is used. When it is desired to bring out structural characteristics, it is advisable to employ solutions which stain more feebly, such as methylene blue or thionin. In either case the simple or reinforced stains, given above, may be employed.

To make a stained preparation of a pure culture the procedure is as follows: A drop of water, preferably distilled, is placed upon a clean cover glass, which either lies on a board or is held in a pair of forceps. By means of a sterile platinum wire a minute amount of the bacterial growth is picked up and transferred to the water. Only enough should be added so as to impart to the water a slight cloudiness. The remainder on the wire is then burned off. The drop is then spread over the whole surface of the glass and allowed to dry in the air, or the process may be hastened by passing it above a flame. The air-dried preparation must now be fixed in order that the bacteria are not washed off in the subsequent treatment. The fixing is done by passing the cover glass three times through a flame. Care must be taken not to scorch the specimen, for in that case the dye will not act. It is well to turn down the flame so that it is at most but two inches high. The properly fixed cover glass is now covered with the stain, which is allowed to act for ten to twenty seconds. The dye is then washed off under the tap and the cover glass inverted upon a glass slide. Any water which may be on the surface of the slip should be removed by means of a piece of filter paper. The preparation can now be examined under a No. 7 objective, or with the one-twelfth inch oil immersion lens. If the specimen is such as to merit preservation a drop or two of water may be applied to the edge, and in this way the slip can be floated off without damaging the film. The excess of water can then be touched off with a piece of filter paper, after which the specimen is dried in the air or by passing it over the flame. The thoroughly dried film is then inverted upon a drop of Canada balsam placed on the centre of a clean slide. By gentle warming or by pressure the balsam can be made to spread out evenly.

Smear or streak preparations made from the fluids or organs of the body are stained in the same way. The fixation of the cover glass when it contains much proteid matter, as in the case of blood streaks, requires special care. The best results are obtained by immersing the slip for a few minutes in a mixture of equal parts of absolute alcohol and ether. Fixation is thus accomplished without any injury to the blood cells. It is sometimes advisable instead of adding the dye to the cover glass to float the latter upon the dye in a watch glass.

To make good blood preparations requires considerable care and experience. A small drop of blood is placed on a perfectly clean cover glass, which is held in a pair of forceps. A second cover slip is then applied, evenly and without pressure, and as soon as the blood has spread out the two glasses are drawn apart. The blood cells must not be crushed and should lie in a single layer.

The smears from the cut surface of an organ are made by gently applying the cover glass, without pressure, and then drawing it away; or a piece of the organ may be taken up in the forceps and streaked over the cover

glass, care being taken to leave only the thinnest film possible.

The ordinary glass slide is often used in place of the cover glass. The streaks or blood smears are made as in the case of the latter. When the growth is hard, as in the case of actinomyces, it is well to crush it between two glass slides.

Double Staining.—This procedure is resorted to when staining the tubercle bacillus and the allied acid-resisting bacilli. It is also used for staining spores, and in differentiating bacteria by means of Gram's stain. Other special methods are given under gonorrhœa and meningitis.

The group of acid-resisting bacilli, the type of which is the tubercle bacillus, are stained with more or less difficulty by the simple stains. The dye, however, can be forced into the cell by the aid of heat, and especially if the reinforced stains, such as carbolic fuchsin or aniline water gentian violet, are used. On subsequent treatment with acid and alcohol the ordinary bacteria, which chance to be present, are readily decolorized, whereas the acid-resisting retain the stain. A contrast color, such as methylene blue, will then stain the background and the ordinary bacteria a light blue.

Staining of the Tubercle Bacillus.—The cover glass is air-dried and fixed in the usual manner, after which any one of several methods may be used. The *Ziehl-Neelsen* method is usually employed. It is carried out as follows. The cover glass, held in the forceps, is covered with carbolic fuchsin and heated over the flame so that vapors are given off for one or two minutes. It is then rinsed in water and dipped for a few seconds in a twenty-per-cent. solution of nitric acid, after which it is washed in dilute alcohol (sixty per cent.) till it is almost colorless. Methylene blue is then applied for a few seconds and washed off. The specimen is transferred to a slide, the surface dried, and examined under the microscope. The tubercle bacilli will appear red on a blue background. The ordinary bacteria will appear blue.

The *Koch-Ehrlich method* consists in staining with aniline water fuchsin or gentian violet with the aid of heat for a few minutes. The specimen is then decolored in thirty-five-per-cent. nitric acid for about a quarter of a minute, washed in dilute alcohol till nearly colorless, after which methylene blue or Bismarck brown is applied for a contrast color.

In the *Fraenkel-Gabbet method* the preliminary staining is effected with carbolic fuchsin as above. The decoloration and contrasting is done at once by immersing the cover glass in a saturated solution of methylene blue in the following: Sulphuric acid 25 parts, alcohol 50 parts, distilled water 1,000 parts. It is then rinsed with water and examined.

Czaplewsky's method differs from the preceding in the way the decoloration is effected. He employed for this a solution of 1 gm. of fluorescein and 5 gm. of methylene blue in 100 c.c. of alcohol. The specimen is first stained with carbolic fuchsin; then, without rinsing in water, it is placed for a few seconds in the fluorescein methylene blue solution. Finally it is dipped ten or twelve times in a solution of 5 parts of methylene blue in 100 parts of alcohol. It is then washed with water and examined.

Numerous modifications of these methods have been proposed, but they possess no special advantage over those given.

Staining of Spores.—The cover-glass preparation is treated for some minutes with hot carbolic fuchsin, either on the forceps or by floating on the dye. It should then be rinsed and examined in water. If the spores are colored the next step is taken; if not, then the heating with the dye is continued until they are stained. The specimen is then decolored in dilute acid and alcohol until the spores stand out red on a colorless background. Methylene blue is then applied for a contrast, washed off, and the preparation is ready for examination. The bright red spores are seen within the light blue cells. This method requires considerable care, and every step must be controlled by frequent examinations under the microscope.

In order to enable the dye to more readily enter the spore, Möller treated the cover glass, first, for a minute or two with a five-per-cent. solution of chromic acid, after which essentially the above procedure was followed. By repeated passage through the flame or by heating with strong sulphuric acid for a few seconds the substance of the spore can be disintegrated so that on subsequent staining with carbolic fuchsin the spores will readily take the dye. This treatment, however, destroys the original cell, and hence contrast coloration is not possible.

The Gram Stain.—This is one of the most valuable methods in bacteriology, since it often serves to distinguish between organisms which otherwise resemble each other very closely. The cover-glass preparation is floated for a few minutes on aniline water gentian violet, or on carbolic gentian violet. Heat may be applied, but in that case the excessive staining will interfere with the subsequent decoloration. The specimen is then rinsed in water and immersed in Lugol's iodine solution for two or three minutes. After rinsing in water it is then placed for a few minutes in strong alcohol until most of the dye has been washed out. Very dilute eosin solution is now applied for about five seconds. After thorough washing with water it is ready for examination. The organism will appear a deep violet on a pink background.

Gram's method is applicable to the bacilli of anthrax, symptomatic anthrax, diphtheria, leprosy, malignant œdema, mouse septicæmia, rouget, tetanus, tuberculosis, the Fraenkel diplococcus, micrococcus tetragenus, the various staphylococci and streptococci, actinomyces, moulds, and yeasts. It is not given by the bacillus of glanders, typhoid fever, hog cholera, Asiatic cholera, chicken cholera, influenza, plague, Friedländer's bacillus, colon bacillus, gonococcus, rhinoscleroma, and recurrent fever spirillum.

The Staining of Flagella.—Special care must be given to the preparation of the cover glass. The cultures should be made on freshly inclined, moist agar, and should, as a rule, be less than twenty-four hours old. A very dilute suspension of the growth is made, and when spread over the cover glass is allowed to dry in the air. The fixation must be done with the least amount of heat possible. This can best be done by passing the cover glass, held between the thumb and forefinger, through the flame.

In Löffler's method the specimen is covered with a mordant solution which consists of 100 parts of a twenty-per-cent. tannic-acid solution, 50 parts of a cold saturated ferrous sulphate solution, and 10 parts of alcoholic fuchsin. The cover-glass is heated over the flame so that vapors are given off for a minute or two. Every trace of the mordant must then be removed by washing with water, and if it has dried down around the edge, it should be removed with a knife. The last traces of the mordant can be removed by momentary immersion in absolute alcohol. The specimen is then heated with aniline water fuchsin for a couple of minutes, washed with water, and examined. The chief difficulty in this method lies in the formation of a heavy deposit of foreign matter, which masks the bacteria.

Fischer's mordant is a slight modification of that of Löffler. It consists of 2 gm. of dry tannin, 20 c.c. of water, 4 c.c. of ferrous sulphate (1:2), and 1 c.c. of concentrated alcoholic fuchsin. The aniline water fuchsin is made by adding about 5 gm. of fuchsin, and 1 c.c. of a one-per-cent. solution of sodium hydrate to 100 c.c. of aniline water.

Bunge employed a mordant consisting of 75 parts of concentrated tannin solution, 25 parts of a five-per-cent. solution of ferric chloride, and 10 parts of a concentrated aqueous fuchsin solution. After standing some days hydrogen peroxide is added until a reddish-brown color is obtained.

Pitfield makes use of a single solution of mordant and dye. Two solutions are first prepared: (1) consisting of 1 c.c. of saturated alcoholic gentian violet and 10 c.c. of saturated aqueous alum; (2) consisting of 1 gm. of tan-

nic acid and 10 c.c. of distilled water. The two solutions are filtered and then combined. The mixture is heated on the cover glass over a flame for about a minute, and then washed off.

Van Ermengem's method is essentially different. The cover glass is warmed for about five minutes with a fixing solution consisting of 60 c.c. of a twenty-per-cent. tannin solution, 30 c.c. of two-per cent. osmic-acid solution, and four to five drops of glacial acetic acid. It is then washed with water, rinsed in alcohol, and dipped for one or two seconds in a sensitizing solution of silver nitrate (one-half to one per cent.). After this it is placed for a few seconds in the reducing solution which consists of 5 parts of gallic acid, 3 parts of tannic acid, 10 parts of sodium acetate, and 350 parts of distilled water. It is again placed in the silver nitrate solution, in which it is moved about until the liquid darkens, after which the preparation is washed with water, dried, and examined.

Of the numerous other modifications which have been proposed that of Gemelli only need be given. Gemelli cleans the cover glasses in a boiling mixture of potassium bichromate (three per cent.), and sulphuric acid (five per cent.). After washing in water they are kept in alcohol. Before use each cover glass is flamed several times. Gelatin cultures developed at 37° C. are said to give the best result. A loopful is transferred to 5 c.c. of water in a watch glass, and from this suspension a drop is taken and spread over a cover glass, which is then set aside over calcium chloride to dry. The specimen is then placed for ten to twenty minutes in a one-fourth-per-cent. solution of potassium permanganate. The preparation is now washed well in distilled water, after which it is placed in a three-fourths-per-cent. solution of calcium chloride (?), to which has been added a one-per-cent. solution of Grübler's neutral red in the proportion of twenty to one. After remaining in this for fifteen to thirty minutes the specimen is washed, dried, and mounted. The method is said to give excellent and sure results without the annoying precipitates which form in the other procedures.

Staining of Capsules.—Welch's method consists in treating the cover glass with glacial acetic acid for a few seconds. The excess of acid is drained off with a filter paper, after which the specimen is washed in aniline water gentian violet, and finally in a sodium chloride solution (0.85 to 2 per cent.). The heavily stained bacillus will be found to be surrounded by a pale violet halo.

Nicolle treats the cover glass with a mixture of one per cent. carbolic acid (100 parts) and concentrated glacial alcoholic gentian violet (10 parts). It is then washed in absolute alcohol containing one-third its volume of acetone, rinsed in water, dried and mounted.

Staining of the Babes-Ernst Granules.—Neisser recommends the following method as a means of differentiating the diphtheria bacillus from like organisms. A culture grown on Löffler's serum should be used. The specimen is treated for one to three seconds, or a little longer, with the following solution: 1 gm. of methylene blue, 20 c.c. of absolute alcohol, 50 c.c. of glacial acetic acid made up to one litre with distilled water. It is then washed with water and stained with Bismarck brown (two per cent. aqueous solution) for three to five seconds. Finally it is washed with water and examined. The blue granules will stand out in the light brown bacilli.

Piorkowski heats the preparation for one-half to one minute with an alkaline solution of methylene blue, then decolors for five seconds in alcohol containing three per cent. of hydrochloric acid. A one-per-cent. aqueous eosin is applied for contrast, after which the preparation is washed and examined.

Romanowsky's Chromatin Stain.—This method is extremely valuable for staining protozoal parasites, such as those of malaria and the trypanosomes. When properly carried out it gives an admirable differentiation of the chromatin, which appears red on a blue background. Nocht's modification gives very good results, and is briefly as follows: A solution of one-per-cent. methylene blue and one-half per cent. of sodium carbonate is kept at about 60° C. for several days to "ripen." The change which takes place is one of slow oxidation, and as a result a number of products form, among which is the one which is essential to this method. This active red constituent has been designated as methylene azur. To about 2 c.c. of water in a watch glass two to three drops of a one-per-cent. solution of eosin are added, and then the altered blue, drop by drop, till the eosin tint just disappears. The specimen is floated on this dye for five to ten minutes, after which it is washed and examined.

Independently Wright, Leishman, and Reuter arrived at a simple modification. The ripened or polychrome methylene blue is treated with an eosin solution to slight excess. The precipitate, which Reuter has called a methylene-blue eosin, is then filtered, washed and dissolved in methyl alcohol. This solution can now be obtained from Grübler. Thirty drops of this are added to 20 c.c. of distilled water in a large watch glass or Petri dish. The specimen, which can be fixed with ether alcohol or with formaldehyde alcohol (10:90), is immersed in the dye for fifteen to thirty minutes. It is well gently to agitate the liquid from time to time. It is then washed, dried, and mounted.

Giemsa prepares the stain by adding 1 c.c. of a 0.08-per-cent. solution of methylene azur hydrochloride, Höchst, to 10 c.c. of a 0.005-per-cent. aqueous solution of Höchst eosin. Laveran employs 1 c.c. of a one-per-cent. solution of azur, 2 c.c. of a 0.1-per-cent. solution of eosin, and 8 c.c. of water. The specimen is stained for ten minutes, then washed and immersed for two to three minutes in a five per-cent. tannic solution, after which it is washed, dried, and mounted.

Impression Preparations of Colonies.—It is very often desirable to reproduce or preserve the characteristic surface colonies. The selection of the surface colony is made under the microscope, after which the tube of the instrument is raised and a cover glass is dropped down upon the colony. Gentle pressure is applied, the cover glass lifted off, air-dried, fixed, and stained with methylene blue in the usual way.

Staining of Sections.—In order to study the finer distribution of bacteria in the body of an infected animal it is necessary to harden portions of the different tissues and organs, which are then cut up into sections. The tissue may be cut by the aid of a freezing microtome, but the best results are obtained when the material is embedded in celloidin or in paraffin. The latter is especially to be recommended. The methods which are used for this work are essentially histological, and need not be considered in this connection.

To obtain a simple stain the section is placed in the dilute aniline dye for about five minutes. Dilute carbolic fuchsin or carbolic methylene blue are very good for this purpose. It is then washed thoroughly in water and transferred to very dilute acetic acid (1 c.c. of glacial acetic acid to 1,000 c.c. of water). The section is now placed in strong alcohol for one-half to one minute to remove the excess of dye. After washing in water it may be examined, and if the decoloration has not been sufficient, the treatment with alcohol can be repeated. When properly differentiated the section is placed for a few seconds in absolute alcohol for dehydration, then cleared in oil of cloves, passed through xylol and mounted in Canada balsam.

In Kühne's method the sections are stained in carbolic methylene blue and differentiated in one-half per cent. hydrochloric acid, rinsed in dilute lithium carbonate solution, then in water. The section is transferred to absolute alcohol, which is slightly colored with methylene blue for one-half minute; then for a few minutes to aniline oil containing methylene blue, finally into clear aniline oil, turpentine, xylol, and balsam.

Nicolle stains the sections in carbolic thionin, washes in water for about a minute, dehydrates with absolute alcohol, clears up in oil of cloves, and mounts in balsam. Another procedure employed by him is to stain first with Löffler's methylene blue, differentiate in one-half per cent. acetic acid, and fix in ten-per-cent. solution of tannin for a few seconds. The section is then washed, dehydrated, cleared, and mounted.

Gram's stain is applied to sections in the following manner: The section is placed in the freshly prepared aniline water gentian violet for about ten to fifteen minutes, after which it is washed in water, or better in aniline water, to remove the excess of dye. The section is then placed in Lugol's iodine for three to five minutes, transferred to absolute alcohol, in which it is tilted about until the excess of the stain has been removed and only a pale violet color remains. Ebner's solution may also be used for decoloring. It is counterstained in very dilute eosin for about a minute, washed in water, dehydrated in absolute alcohol, cleared in cloves, and mounted.

Bismarck brown may be used for a contrast color, or Weigert's picrocarmine. The latter may be made by adding 1 part each of carmine and ammonia to 50 parts of water; to this solution picric acid is added until a precipitate forms which is dissolved by the addition of a little ammonia; finally a few drops of carbolic acid are added.

Tubercle and leprosy bacilli can be stained in sections by applying the principle employed for cover-glass preparations. The carbolic fuchsin should be warmed to about 40° C. in a Petri dish. The sections remain in this solution for fifteen to thirty minutes, after which they are washed in water to remove the excess of dye. They are then decolored in dilute acid, or better in Ebner's solution. The latter consists of one-half part each of sodium chloride and hydrochloric acid, 30 parts of water, and 100 parts of alcohol. The faint pink sections are then placed in Löffler's methylene blue solution for about half a minute, after which they are dehydrated in absolute alcohol for a few seconds, transferred to xylol, and mounted in balsam.

Diagnostic Methods.—The general principles which have been given find their practical application in the diagnosis of disease. It is desirable, therefore, to indicate briefly the way in which these methods are applied.

1. *Actinomycosis.*—The pus should be examined unstained under a cover glass for the characteristic yellowish, radiating masses with club-shaped threads along the border. Permanent preparations may be made by making smears, and staining either by the simple or by the Gram method. The pus may be hardened in mercuric chloride, sectioned and stained by Gram. Curettings may be treated in like manner.

2. *Anthrax.*—Cover-glass smears of the blood are stained by the simple and by the Gram method. Confirmation of the nature of the organism found can be obtained by inoculating a white mouse or a guinea-pig subcutaneously with the material. This with the cultural characteristics will enable identification.

3. *Bubonic Plague.*—This diagnosis may be made during life, but more often after death. In the pneumonic form the blood-streaked sputum can be used for simple stains, which will show large numbers of the small rods. In the bubonic type the enlarged gland may be punctured and thus material obtained for examination. The detection of the bacillus in the blood can be effected by drawing several cubic centimetres of the blood from a vein by means of a syringe. Agar plates, one part of blood to two parts of agar, should then be made. Whether the result is apparently positive or wholly negative, an animal experiment must be carried out. The suspected material is inoculated subcutaneously into a guinea-pig, and if plague bacilli are present, death will ensue in from five to eight days. Cultures and smears from the animal will then establish the nature of the organism. An important cultural characteristic is brought out by planting the material on agar containing about three per cent. of salt. Round or pear-shaped involution forms develop. The smears from the body should be stained with Löffler's methylene blue or with carbolic thionin. The short rods take the bipolar stain, and if the material is old, roundish involution forms or "doughnuts" will be met with. The bacilli are not stained by Gram.

4. *Cholera.*—The examination may aim to find the cholera vibrio in the drinking-water, or it may be concerned with the diagnosis of the disease. In the latter case the rice-water discharges should be collected and searched for mucous flakes. Preparations from these, when stained, will show the presence of the characteristic vibrio. The appearance of the colonies on gelatin and on agar plates, the growth in bouillon, and the indol reaction serve to identify the organism. The intraperitoneal injection of the culture into a guinea-pig should also be practised. The most important reaction is that of Pfeiffer. It consists in injecting into the peritoneal cavity of a guinea-pig a mixture of the cholera antiserum and the suspected organism. Every few minutes a drop of fluid is withdrawn from the peritoneal cavity by means of a capillary tube and examined. If the organisms persist, it is safe to say that they are not those of true cholera. The latter under these conditions lose motion, become granular, and soon disappear.

It may be possible under exceptional circumstances to detect the cholera vibrio in drinking-water by ordinary plating on gelatin. Obviously the number of the organisms may be so small and the other bacteria may be so numerous that it is impossible to obtain positive results by this method. To overcome this difficulty Schottelius devised his enriching method. This consists in adding the suspected water to a one-per-cent. solution of peptone and incubating at 37° C. for about twelve hours. The actively motile cholera spirilla, on account of their need of oxygen, accumulate as a cloudy layer near the surface. A loop of this liquid is then transferred to and spread over the surface of gelatin and agar plates, and the further identification is then easily effected. Obviously the peptone may be added direct to the water, for example 100 c.c., and in this way the presence of a very few vibrios in a large volume of water may be detected.

5. *Diphtheria.*—The necessary material for the examination is obtained either by means of a Roux spatula (Fig. 5083), or by a cotton swab. The cotton swab is usually employed, and is made by twisting a piece of cotton about the end of a thick iron wire. The wire should be about six inches long. The cotton end is then placed in a plugged test tube which is sterilized by dry heat. Whether a pseudo-membrane is present or not, scrapings are made from the surface of the affected tonsils or throat and examined. Usually the swab is streaked over the surface of one or more tubes of plain, or better Löffler's serum. These tubes are incubated overnight and examined in the morning for the characteristic diphtheria bacilli. The cover-glass preparations made from these cultures are stained with Löffler's methylene blue. The swollen rods with irregularly stained contents are easily identified. In case there is any doubt as to the diagnosis, it is advisable to inoculate a guinea-pig with the culture material.

Whenever possible it is advisable to make cover-glass preparations direct from the false membrane. These are then stained with Löffler's methylene blue. The diagnosis can be made thus often in a few minutes.

6. *Dysentery.*—It is necessary to distinguish between two types, the bacillary and the amœbic. The examination for amœbæ should be made at once, immediately after the stool is passed, in order to obtain actively motile organisms. A drop of the thin fæces or suspension is placed on a slide, covered with a cover glass, and examined under the microscope. The characteristic motion will leave no doubt as to the nature of the organism. The motion can be observed best on a warm side or in an incubator. Staining is not necessary.

The bacillary form is due to the bacillus dysenteriæ, and the diagnosis of this type necessitates the detection of this organism. Agar plates should be made from the stools and developed at 37° C. From the suspected colonies subcultures should be made to glucose agar, lactose litmus agar, and mannite litmus agar. The pure cultures should finally be tested for agglutination with the serum of a dysenteric patient. Too much care, however, cannot be exercised in drawing conclusions based upon a

positive agglutination reaction, since this test is undoubtedly given by allied organisms.

7. *Gonorrhœa.*—Cover-glass preparations made from the pus will serve to establish the diagnosis in nearly all instances. They should be stained with Löffler's methylene blue. A fair double stain can be obtained by first applying eosin, after which the blue can be used for a few seconds. The result is a more or less pink background with blue gonococci. Gram's method is negative.

Von Wahl recommends the following method of double staining which brings out the gonococci as reddish-violet to black cells on a light green background. The stain consists of: Concentrated alcoholic solution of auramin, 2 c.c.; ninety-five-per-cent. alcohol, 1.5 c.c.; concentrated alcoholic solution of thionin, 2 c.c.; concentrated aqueous methyl green, 3 c.c.; water, 6 c.c. The auramin and thionin solutions are prepared by dissolving the dyes in hot ninety-five-per-cent. alcohol to saturation, cooling, and filtering. The cover glasses are stained for five to fifteen seconds. The ordinary bacteria stain feebly or not at all.

The detection of the gonococcus in septicæmic cases can be accomplished by drawing 5-10 c.c. of blood from the vein of the arm and adding this, in about equal parts, to melted agar at 45°. The mixture is at once poured into Petri dishes, and these are developed at 37° C. In this way the gonococcus can be detected when direct stains would fail to show the organism.

The culture test for the gonococcus is rarely resorted to on account of the difficulty of obtaining the necessary blood or serous fluids. The ordinary media have always been regarded as unfavorable for the growth of this organism. According to Thalmann, Wildbolz, and others, the gonococcus can be grown on ordinary one-and-one-half-per-cent. meat peptone agar. Thalmann recommends very highly such a medium for diagnostic purposes, and especially where direct microscopic examination is unsatisfactory or negative. The acidity of ordinary agar or bouillon is reduced by the addition of two-thirds of the amount of alkali necessary to make the media neutral to phenolphthalein. The preparation of these media has been given.

8. *Leprosy.*—The leprous nodules are characterized by the presence of enormous numbers of the specific bacillus which can be readily detected by staining. The Ziehl-Neelsen method, as employed for the tubercle bacillus, will give excellent results if the tissue is reasonably fresh. When kept for some time in alcohol the bacilli lose their staining properties so far as this method is concerned, but they can still be found by means of Gram's method. Animal inoculations and cultures are not possible.

9. *Meningitis, Cerebro-spinal.*—The diplococcus intracellularis meningitidis is found in the cerebro-spinal fluid. Hence during life it is necessary to remove some of the fluid by lumbar puncture. This fluid should be planted abundantly on glycerin, or better on serum agar. Cover-glass preparations made direct will show the typical organism resembling the gonococcus in form and in its presence within the leucocytes. It is not stained by Gram's method, but can be given a double stain by that of Pick and Jacobsohn, or by the modification suggested by Fraenkel. The dye is made by adding to 20 c.c. of water eight drops of a saturated solution of methylene blue, and then forty to fifty drops of carbolic fuchsin. The dye is allowed to act for five minutes. The cocci are blue on a red background.

10. *Pneumonia.*—In all pneumonic conditions the blood-streaked sputum should be examined by making simple and Gram stains. In this way it becomes possible to recognize the pneumonic form of plague. Ordinarily, however, pneumonia is due to the Fraenkel diplococcus and at times to the Friedländer pneumobacillus. The form, staining and cultural properties of these organisms permit ready differentiation and identification. The lance-shaped diplococcus of Fraenkel, as found in the body, is surrounded by a capsule, and is stained by Gram's method. The colonies and cultures on glycerin agar are very faint and dewdrop-like, and tend to die out in a few days. Their vitality and virulence are best preserved by cultivating them on rabbit blood or serum agar. In doubtful cases the material should be injected under the skin of the ear of a rabbit. If death results the diplococcus will be found in large numbers in the heart blood and organs of the animal.

11. *Rabies.*—The cause of this disease is as yet unknown, but it is to be found, in pure condition so to speak, in the brain and spinal cord of the affected person or animal. The diagnosis rests upon animal inoculation with such material. A few drops of a suspension of the brain or cord are injected subdurally into a rabbit or guinea-pig. The method has been already described.

The histological changes in the nervous system are very slight, and it has been suggested that the diagnosis of rabies may be hastened by making an examination of sections of the cord and ganglia. The lesions are not sufficiently marked in all cases to permit diagnosis, and for that reason this method should not be relied upon to the exclusion of the only positive test, that of animal inoculation.

12. *Tetanus.*—The point of inoculation must be found first. This may not always be easy, for the original wound may have healed over. The portal of entry may be a bad tooth, or the wound produced by an old rusty nail, a splinter of wood, or the powder grains of a pistol. Cover-glass preparations should be made from the pus, if there is any; and, if not, from such serum, blood or tissue as can be obtained from the wound. They should be stained with carbolic fuchsin. The specimen should be examined for "drum sticks" or rods with terminal spores, and particularly for the presence of rather narrow, long bacilli. These are present even when no spores can be found.

Cultures should be made on glucose litmus gelatin, and at the same time a series of glucose agar plates should be made and developed at 37° C., either in hydrogen or in the pyrogallate apparatus. The original tissue should be planted under the skin of a white mouse and of a guinea-pig. The cultures when developed are carefully searched for the drum-stick forms.

13. *Tuberculosis.*—The pulmonary form is usually recognized by an examination of the sputum, preferably that which has been collected in the morning on rising. The cheesy particles, if such can be recognized, should be picked out by means of the wire and spread over the cover glass. The specimen is then stained by the Ziehl-Neelsen method already given. The red rods on a blue background are readily recognized.

The same method is employed in the examination of pleuritic fluid, pus, urine, milk, etc. In all these examinations, however, two facts should be borne in mind. In the first place the tubercle bacilli may be present, but in such small numbers that they escape detection. Again, acid-resisting organisms, such as the leprosy, smegma, timothy grass bacillus, etc., may be present, and may be easily mistaken for the tubercle bacillus.

In either case it is the animal experiment which will serve to remove the doubt. When the tubercle bacilli are few or doubtful, it is well to submit the material to preliminary centrifugation. The deposit can be used then for staining and for injection. The injections should be made into the peritoneal cavity of the guinea-pig. If tubercle bacilli are present in the material used, even if so scarce as to be unrecognized by the microscope, the animal will develop the disease and will die in the course of a month or two. The tuberculous organs and glands can be examined then for tubercle bacilli, and cultures can be made on glycerin agar, potatoes, and on Hesse's Heyden agar. The acid-resisting bacilli, other than the tubercle bacillus, are not fatal to animals, and, moreover, the histological changes which they induce are quite different from those caused by the tubercle bacillus.

14. *Typhoid Fever.*—The verification of the diagnosis is usually made by means of the agglutination test of Widal, which will be described later. The direct detection of the typhoid bacillus in fæces, urine, blood, rose spots, and in water presents marked difficulties. The

reason for this lies in the very great similarity which exists between the typhoid and the colon bacillus. Numerous methods have been devised for the purpose of effecting a distinction between these two organisms; and while it is an easy matter to differentiate between the pure cultures of the typical organisms it becomes vastly more difficult under natural conditions, especially when, as often is the case, the para-colon and para-typhoid bacilli are present.

Some of these methods endeavor to restrict the growth of the colon and of adventitious bacteria by the addition of antiseptic substances to the media. Thus carbolic bouillon, Parietti's carbolic hydrochloric-acid broth, and crystal violet are used with this object in view, but there can be no doubt that weak typhoid bacilli are also inhibited.

Other methods are based upon the unequal diffusion of the two organisms in special media. The Stoddart, Hiss, and Capaldi media belong to this class. Again, the effort is made to bring out differences between the colonies of the Eberth and colon bacilli, as in the case of the Holz potato gelatin, Elsner, Weil, Hiss, and Piorkowski media. These are all described in the foregoing pages. Still other methods seek to utilize the differences in the fermentative powers of these organisms, as evidenced in the production of gas, acid products, etc. Lastly, there are several methods which have only recently been devised in which the distinction is effected by means of the agglutination reaction. That of Windelbandt, as modified by Schepilewsky, certainly seems to be effective in detecting typhoid bacilli in tap water. Its real value in the examination of typhoid fæces remains to be demonstrated.

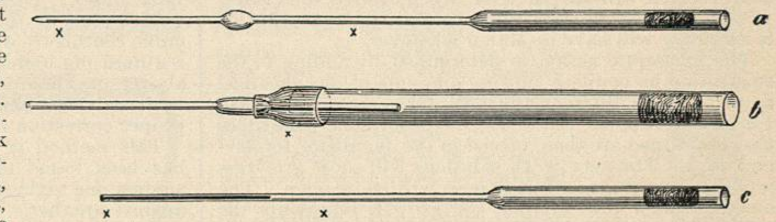

FIG. 5109.—Filling of Capillary Tubes for Thermal Death-point Determinations. *c*, Tube filled ready to be sealed at x; *b*, emptying of tube. (Novy.)

Schepilewsky's procedure is as follows: Several cubic centimetres of the infected water are added to 50 c.c. of bouillon in an Erlenmeyer flask, which is then incubated for twenty-four hours at 37° C. The culture is now filtered through a sterile cotton filter in order to remove any compact masses of bacteria which may be present. The filtrate is received in conical centrifugating tubes. A very active serum from a rabbit, which has been immunized to the typhoid bacillus, is then added to the cloudy filtrate, and this is set aside for two to three hours at 37° C. If many typhoid bacilli are present, visible agglutination may be noted; but if they are not abundant, the masses will be so small that agglutination may not be detected. In either case the tubes are centrifugated for one to two minutes, after which the fluid is decanted and the tubes are inverted so as to drain as completely as posssible. Sterile sodium chloride solution is then added to the tubes and the deposit is taken up and transferred to a sterile test tube which contains glass beads. By vigorous shaking the agglutinated masses can now be broken up and homogeneous suspension obtained. A glass rod, bent at right angles, is then dipped in the suspension, and streaked thoroughly over the surfaces of a number of plates of the special agar. The latter is a three-per-cent. agar, to which after sterilization in an autoclave a lactose and lacmoid solution is added. This consists of 1.5 per cent. lactose and 0.04 per cent. lacmoid, and before addition is boiled fifteen minutes. The Petri dishes are developed at 37° C. for twenty-four hours. The typhoid colonies are round, or oval, and sharp bordered; later they show the typical spreading form, and the color of the centre changes to a dirty yellow.

The typhoid-like colonies should then be examined under the microscope and submitted to further identification. A suspension of the colony may be tested for the agglutination reaction with typhoid serum. Pfeiffer's reaction may be tested for by injecting a mixture of antityphoid serum and the organism into the peritoneal cavity of a guinea-pig, as described under Cholera. The cultures should also be tested for gas production, indol, milk coagulation, and on the special plating media.

In the absence of the serum necessary for the above method recourse may be had to the plating medium of Hiss, the urine gelatin of Piorkowski, and the Drigalski-Conradi agar, the preparation of which has been given.

15. A number of infections due to moulds and allied forms and also to yeast-like bodies have been described. The former are represented by the streptothrices, or, more correctly, by the actinomyces of Madura foot and of cattle farcy. The yeast or blastomycotic affections have been noted in certain forms of dermatitis, and may even be systemic in character. In all these diseases the examination of the pus and of sections of tissue, as well as the culture of the organism, must be carried out.

16. *Protozoal Diseases.*—Several very important diseases are due to organisms of this class. The examination for amœbæ in tropical dysentery has already been touched upon. The sporozoa include among others the plasmodium of malaria, the piroplasma of Texas fever, of "spotted fever," and of horses, sheep, and dogs. The flagellata cause the various trypanosomatic diseases, such as the surra of Asia and of the Philippine Islands, nagana or the tsetse-fly disease of South Africa, dourine or "maladie du coït" of the Mediterranean littoral, caderas of South America, the gambian fever and the sleeping-sickness, the last two being diseases of man. Moreover, many animals harbor in the blood parasites of this group, as in the case of the wild rat.

In all suspicious cases the blood should be examined for these two groups of organisms. The plasmodium of malaria is found especially within the blood cells. The trypanosomes are free in the plasma. The blood may be examined direct under the cover glass, or in hanging drop, or in a Ranvier slide. Stained preparations can be made with methylene blue, thionin, or best with some modification of the Romanowski method. The preparation of the specimens and the staining methods have already been described. The trypanosomes of the rat and of nagana, cultivated by Novy and McNeal, are the only pathogenic protozoa which it has been possible to grow artificially. Their presence is best detected by direct examination of the blood, though at times they may be very difficult to find, and may require daily examinations for several weeks.

DETERMINATION OF THE THERMAL DEATH POINT OF BACTERIA.—In this work and also in testing disinfectants it is necessary to have almost homogeneous suspensions of the organism to be tested. Water suspensions should be used first, and later those of bouillon, serum, etc. The liquid is introduced by means of a bulb pipette into the tube of an agar culture. The growth is rubbed up as much as possible, and the suspension is then transferred to a sterile glass-wool filter. In this way the masses of bacteria are removed. The cloudy filtrate may be used as such, or it may be diluted so as to have fewer organisms in the test. It may be used as such for determining the action of moist heat and for testing disinfectants. Again, for dry-heat work and for many disinfecting tests the suspension is allowed to dry upon the surface of sterile glass slips, glass or garnet beads, silk threads, muslin squares, etc.

To test the action of dry heat a number of cover glasses on which the test organism has been dried are placed in a sterile Petri dish and exposed to a given temperature.

At given intervals a specimen is removed and planted in bouillon.

To test the action of moist heat the best procedure is to draw up the liquid into sterile capillaries, as shown in Fig. 5109. The tube is sealed, below and above, the liquid. The advantage of this method lies in the fact that the heat promptly penetrates every part of the suspension. A number of tubes thus equipped are placed in a water-bath, the temperature of which is kept at a constant point by means of a regulator. At intervals a capillary is removed, cooled, opened at one end, and the contents are expelled into a tube of bouillon by gently touching the closed end to a flame.

Testing of Chemicals.—In this work it is necessary to distinguish between the antiseptic and the disinfecting action of a substance. The former refers to the amount of the substance which will inhibit the growth, but not necessarily kill the organisms. The latter implies the actual destruction of the test object. Obviously a substance which will kill bacteria, when diluted sufficiently will merely restrict their growth; and when the dilution is excessive will have no action whatever.

The antiseptic action is determined by adding to the suspension in bouillon varying amounts of the chemical so as to make different dilutions, for example: 1 in 500, 1 in 1,000, 1 in 5,000, 1 in 10,000, etc., solutions. The tubes thus equipped are then placed in the incubator for several days. The very weak solutions will show growths, while the very concentrated ones will show none. The amount which just inhibits the growth represents the antiseptic power of the substance.

The germicidal action of a gas, such as formaldehyde, is determined by exposing cover-glass preparations, silk threads, bit of muslin, etc., impregnated with the suspension, dry and moist, to the action of the gas in a tight room. At the end of the exposure the preparations are transferred to sterile tubes of bouillon and incubated.

The germicidal action of liquids is ascertained, either by adding the solution to the bacterial suspension or by immersing in the solution the dried cover-slip preparations mentioned. In the former case, at stated intervals, a small loop of the liquid is transferred to bouillon, while in the latter case the slip or thread is first rinsed in sterile water and then placed in the bouillon.

The Serum Agglutination Test.—The serum of an animal which has been immunized to a germ when brought into contact with a suspension of that germ will cause the organisms to gather in masses. This fact is utilized in the Widal test for typhoid fever. A drop of the serum from a typhoid patient is diluted with ten, twenty, thirty, fifty, one hundred drops respectively of water in a watch glass. A drop of each mixture is then transferred to a cover glass and inoculated with a very small amount of a fresh agar culture of the typhoid bacillus, care being taken to avoid an excess of the organisms. Hanging drops are then made and the preparations examined under the microscope. The limit of the reaction is indicated by the dilution which is just able to cause paralysis of motion and clumping in one hour. Instead of diluting with water some prefer to use a very young bouillon culture. The agglutination test is most delicate when carried out under the microscope. Very good results, visible to the unaided eye, may be obtained by adding the serum to bouillon cultures of the organism. The tubes thus treated should be set aside for some hours at 37° C. when the agglutinated masses will settle to the bottom and leave the liquid clear. When applying the test to a suspected case of typhoid fever it is not always possible to obtain large amounts of the serum. In such instances the blood may be placed in single drops on a sheet of filter paper, or better tinfoil. The dilutions can then be made with these drops of dried blood as with the serum itself. The application of this test to the recognition to the typhoid bacillus in drinking-water, fæces, etc., has been given.

Testing the Strength of Antitoxin.—The strength of an antitoxin is expressed in immunity units. A unit represents that amount of serum which will be just sufficient to save a 250-gm. guinea-pig against 100 minimum fatal doses of the diphtheria toxin. Thus if 0.1 c.c. of serum protects against this dose of poison, then it will contain 1 immunity unit, and 1 c.c. of such serum will contain 10 immunity units. It is possible to prepare diphtheria antitoxin of such strength that 1 c.c. will contain more than 1,000 immunity units. Usually, however, the serum as marketed contains about 200 immunity units per cubic centimetre.

The first essential is to ascertain the minimum fatal dose of the toxin, by which is meant the amount of toxin which will kill a 250-gm. guinea-pig on the fourth, or at most on the fifth day. The toxin itself is a bouillon culture of the diphtheria bacillus, which has been rendered sterile by the addition of a small amount of carbolic acid. The diphtheria culture should not be more than a week old.

Varying amounts of the serum are then added to portions of the toxin representing one hundred minimum fatal doses. These mixtures are then injected into guinea-pigs of the weight given. That fraction of a cubic centimetre of the serum which just suffices to save a guinea-pig under these conditions represents, as stated above, one immunity unit. The test is usually made with only ten minimum fatal doses, in which case the proper correction is made.

This method is subject to some error, inasmuch as it has been found that a serum which has been tested against one toxin will show a different value when tested against another. This is due to the fact that the toxin undergoes changes on keeping, and is converted into a non-poisonous body or toxoid, which, however, retains the power of combining with the antitoxin. For this reason Ehrlich proposed a new method of testing the antitoxic value of a serum. A standard dried antitoxin is made the basis of the measurement. This is diluted so that a given amount just represents one immunity unit. The test dose of toxin is then ascertained and represents the amount of toxin, which mixed with one immunity unit of serum, will cause death on about the fourth day. The serum to be tested is then diluted, mixed with the test dose of the toxin, and injected into guinea-pigs. If the animal dies in a day or two it is evident that the serum contains less than one immunity unit. If, on the other hand, it lives for seven or eight days, or even recovers, it shows that the amount of serum taken contains more than one unit. By using suitable dilutions of the serum eventually the point will be reached where the amount taken will represent exactly one unit.

Methods of Immunization.—Only the general principles of active immunization can be considered. The injections are made subcutaneously, intraperitoneally, and intravenously. When a soluble poison is injected, a true antitoxin develops in the animal and is present in the blood, and hence in the serum. This is the case in diphtheria, tetanus, and venom immunity. On the other hand, when the solid cell is injected, the serum may acquire anti-infectious properties, the best instance of which is seen in the anti-pest serum. The organisms may be killed by exposing them to the action of ether, chloroform, or to that of moist heat at 60° C. In special cases the attenuated living germ is used, and at times even the most virulent form is employed. By the injection of cells other than bacteria, diverse cytolytic sera are obtained. Thus the injection of the red blood cells gives rise to a hæmolytic serum. The temperature and the weight of the animal must be taken daily, since they afford the best indication of the condition of the animal.

Examination of Air.—The bacteria which chance to be present in the air are in a dried condition, and tend to settle when the motion of the air is lessened. The simplest method consists in exposing a plate of gelatin or agar to the air for a given length of time. Some of the organisms settle on the gelatin, and eventually give rise to colonies. Koch improved slightly upon this by placing the gelatin plate in the bottom of a sterile cylinder of known volume. After opening the cylinder in a given locality it is closed with a cotton plug and set aside, when the organisms contained in that volume of confined air settle to the bottom on the surface of the plate. The results, it will be seen, are crudely quantitative (Fig. 5110).

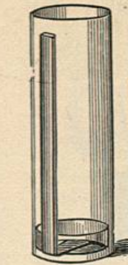

Fig. 5110.—Koch's Cylinder for Air Analysis.

Hesse's method consists in drawing the air through a large Esmarch roll tube (Fig. 5111). The volume of the air drawn through can be determined from that of the aspirating bottles. The bacteria in the air impinge upon the gelatin surface where they develop into colonies which can be counted and studied.

The apparatus of Petri, although very expensive, may be said to give the best results. It consists in the first place of an air pump, which automatically registers the movement of the piston, and thus gives the volume of the air which has been drawn through. The air is aspirated through a tube (Fig. 5112) containing several layers of sterile sand. The suspended bacteria are thus held back. At the close of the operation the sand is transferred to a Petri plate, where the bacteria will form colonies and thus become accessible for study.

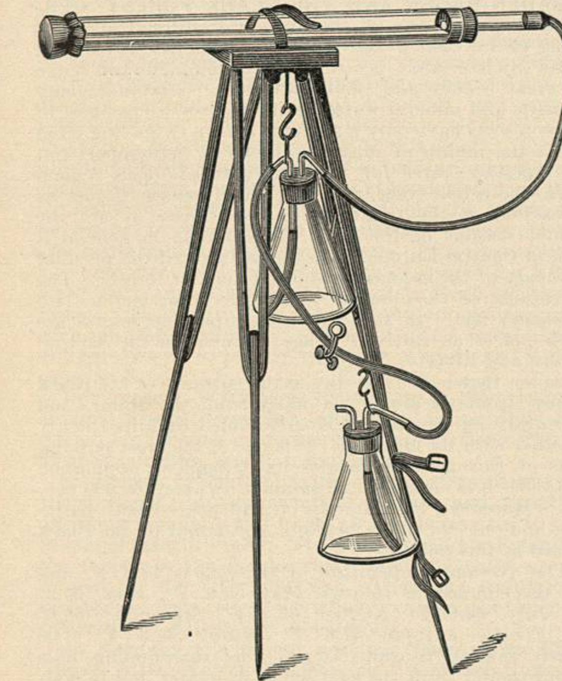

Fig. 5111.—Hesse's Apparatus for Air Analysis.

Instead of sand, Sedgwick and Tucker employed a filter of sugar. The special tube employed by them is shown in Fig. 5113. After the air has been drawn through, the sugar is tapped down into the wide portion of the tube, then gelatin is added and warmed until the sugar dissolves, after which an Esmarch roll culture is made in the same tube.

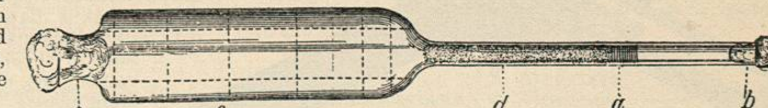

Fig. 5113.--Sedgwick and Tucker's Aërobioscope.

Other workers have filtered the air through liquid media or through gelatin. The most convenient form of apparatus of this kind is that of Wurtz, shown in Fig. 5114. A suitable amount of gelatin is placed in the sterile tube, then a known volume of air is drawn through, after which the gelatin is solidified over the inner wall of the tube, thus forming a roll culture.

Examination of Soil.—By means of a small sterile platinum spoon a definite volume of the soil may be transferred to a Petri plate, where it is thoroughly mixed with the medium. The colonies which develop can then be examined. In this way it is possible at times to demonstrate the presence of the anthrax bacillus in the soil of an infected locality. The detection of other pathogenic bacteria, as for instance those of tetanus and malignant œdema, can best be made by introducing a quantity of the soil under the skin of a guinea-pig or rabbit.

Examination of Water.—This is a very important procedure, and an enormous amount of work has been done to perfect the methods of work. The method followed will necessarily depend upon the immediate object in view. Thus the detection of the cholera vibrio is carried out in a different way from that used for the colon bacillus. The isolation of the cholera and typhoid organisms from water has already been described.

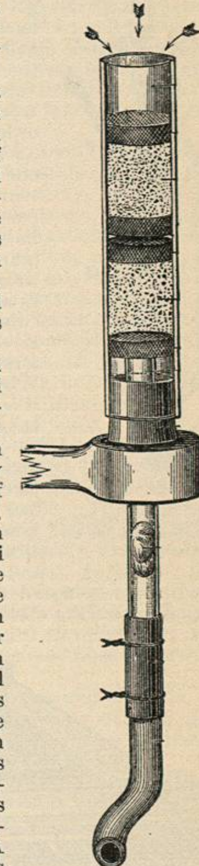

Fig. 5112.—Petri's Sand Filter for Air Analysis.

The water which is to be tested bacteriologically must be received into a sterile glass-stoppered bottle, and should be examined at once. The first step in the examination is to make gelatin plates. By means of a sterile pipette 1 c.c. of water is added to a tube of gelatin, mixed thoroughly, and the gelatin is then poured out into a Petri plate. In the same way plates are made with 0.5 c.c. and with one drop of the water. The gelatin plates are developed at 20° C. for several days. The colonies are then counted and examined in the usual way. When only a few colonies are present on a plate they can be readily counted with the eye. When the number is large special counters are made use of. That of Wolffhügel (Fig. 5115) is ruled in squares of 1 cm. and fractions thereof. Another form is that of Lafar. A very useful and cheap substitute is made by printing the divided circle on card paper. To make a count, the number of colonies in ten or more squares are ascertained and the average per square centimetre is obtained. The area of the gelatin on the plate is taken and then the total number of colonies on the plate determined, and the result is expressed per cubic centimetre of water.

Instead of using Petri plates, the Esmarch roll tube can be made. To count the colonies in such a tube Esmarch devised the counter shown in Fig. 5116.

When the number of colonies is likely to be extremely numerous, as in the examination of polluted water, it is advisable to dilute a portion of the sample with a known volume of sterile water. If the colonies are very nu-

merous on a plate the counting can be carried out best under a microscope. Ehrlich stops are placed in the eye-piece or the special Ehrlich ocular may be used. The size of the opening in the ocular must be determined by means of a stage micrometer. The average number of colonies for a given sized opening is determined, and from this the total number on the plate is calculated.

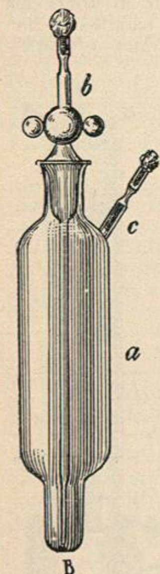

FIG. 5114.—Wurtz's Apparatus for Air Analysis.

The above method of examination gives approximately the number of bacteria which are present in a water, and to some extent information as to the kind of bacteria. It is of great value, therefore, in controlling the work done by the water filters. In order to ascertain the presence of pathogenic and other bacteria special methods must be resorted to. The method which has been employed during the past fifteen years in the Hygienic Laboratory of the University of Michigan consists in planting a cubic centimetre and a drop of the water in tubes of bouillon, which are then incubated at 39° C. If no growth forms, as often is the case, pathogenic bacteria may be said to be excluded. When a growth does form, a portion (1 c.c.) is injected into a guinea-pig. In case the animal dies the heart blood is examined for the kind of organism present. If no ill effects follow the injection, the water may be said to be free from pathogenic bacteria.

Sewage contamination is usually indicated by the presence of colon bacilli and of streptococci. The presence of the former is tested for by means of the Smith fermentation tube (Fig. 5117); 1 c.c. of the water is added to

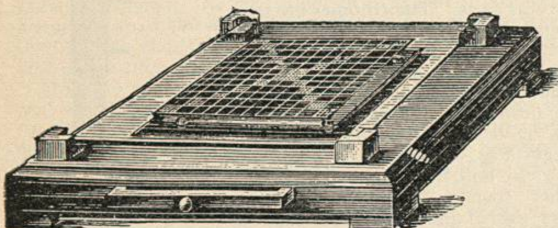

FIG. 5115.—Wolffhügel's Colony Counter.

glucose bouillon in the fermentation tube, which is then set aside at 37° C. The formation of gas indicates the

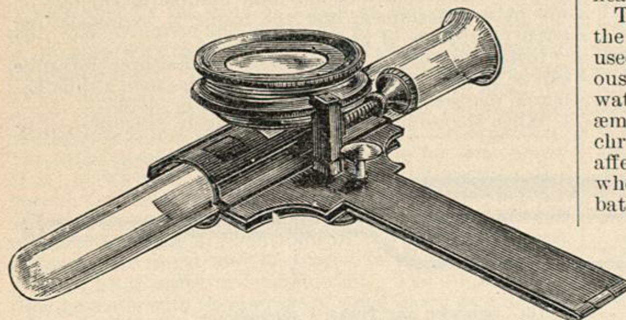

FIG. 5116.—Counter for Esmarch's Roll-Tube Cultures.

probable presence of the colon bacillus, while the non-production of gas points to the absence of this organism. Further tests are necessary for identification.

Litmus lactose agar plates should be made directly from the water and also from the fermentation tube when gas production is present. The formation of red colonies is indicative of the colon bacillus, since this organism ferments lactose, whereas typhoid-like bacteria do not. For the same purpose lactose bouillon is sometimes employed in the fermentation tube.

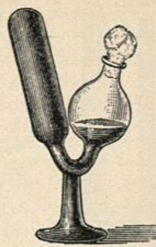

FIG. 5117.—Smith's Fermentation Tube.

Another procedure is to plant the water in carbolic bouillon, or in Parietti's solution, in order to eliminate many of the more common bacteria.

Neutral red bouillon and agar is also used for cultivating the suspected colon bacillus. The water may be planted direct, or the red colonies which form on the plate may be used for inoculation. The coagulation of milk and the production of the indol reaction are additional tests of identification.

F. G. Novy.

BADEN-BADEN AND THE BLACK FOREST, GERMANY.—The region of the Black Forest (Schwarzwald) lying to the east of the Rhine, between Karlsruhe and Basel (Switzerland), is a thickly wooded mountain range of great beauty and picturesqueness, containing many climatic and mineral-water health resorts. The most of these resorts have only a summer season extending from about the middle of May to the end of September; but the open-air cures for pulmonary tuberculosis remain open the whole year, and the winter season is just as efficacious in the treatment of this disease as are the milder seasons of the year. The climate in general is that of Central Europe, with the addition of the peculiar influence of the mountains and the thick fir forests. The elevations of the climatic resorts are from one to two thousand feet. In this country comparable resorts are such places as Rutland, Mass., Saranac in the Adirondacks, and Liberty, N. Y.

Baden-Baden (650 feet) lies at the entrance of the Black Forest from the north, six miles from the Rhine, and is beautifully situated; it is surrounded by hills thickly wooded with the dark fir. It is one of the most popular spas of Europe, and, besides its permanent population of 15,000, it is visited in the summer by about 60,000 people. Moreover, the environs of Baden are most charming, and one can either begin or end a tour of the Black Forest at this point.

The average temperature of the year is 48.3° F., and for the summer as follows: May, 53.9° F.; June, 60.2° F.; July, 62.9° F.; August, 62.2° F.; September, 56.6° F. For the autumn, 47.6° F.; winter, 34.3° F. The yearly rainfall is quite large. The surrounding hills afford shelter from the cold winds in winter, but in midsummer they prevent the cool breezes from mitigating the heat which, for some persons, is too great for comfort.

The waters contain sixteen grains of common salt to the pint, at a temperature of 110° to 154° F. They are used for drinking, but chiefly for bathing, and in various forms of douches. The maladies for which such waters are recommended are rheumatism, gout, lithæmia, gastric catarrh, catarrh of the bladder, scrofula, chronic metal poisoning, obesity, and chronic catarrhal affections of the respiratory tract. The effect of the waters when drunk is diaphoretic, diuretic, and laxative. The bath establishments are extensive and well appointed, some of them luxuriously so, and include hot-air, vapor, pine, electric, and mud baths, and swimming pools. There is also an inhalation establishment, and a section for Swedish massage and mechanical gymnastics. There is an elaborate and extensive *Conversations-Haus* with pleasant grounds, and near at hand is a *Drink-halle,* most frequented in the early morning, when the waters are drunk to the accompaniment of music, the usual custom in European spas. There are also several private sanatoria under competent direction. The sanitary condition of the city is satisfactory, and the water supply for domestic purposes comes from mountain wells. The accommodations are abundant, excellent, and of varying price. The baths are open all the year, although they are most frequented during the season (May to October). There are also milk, herb, and grape cures. The situation of Baden with its hills affords opportunity for the "Terrain-Cur." One can also make an "after-cure" here after a course of more active mineral waters.

East of Baden-Baden, in the northeastern part of the Black Forest, is Wildbad (1,410 feet), a much-frequented spa, containing indifferent thermal waters, of a temperature of 91.3° to 104.5° F., used very largely for bathing, although they are also used for drinking and gargling. The town, of about 3,000 inhabitants, lies in the narrow wild valley of the Enz, surrounded by hills covered with pines, and possesses an agreeable climate and excellent drinking-water. The mean yearly temperature is 46.5° F., and the mean monthly temperatures for the season are: May, 53.7° F.; June, 60.8° F.; July, 63.1° F.; August, 62.6° F.; September, 55.9° F. The waters are beneficial for the class of cases for which simple hot baths are appropriate, such as chronic rheumatism, gout, chronic joint pains, metal poisoning, scrofula, functional nervous affections, catarrh of the respiratory organs, nervous dyspepsia, convalescence from acute and chronic diseases, certain gynæcological affections, and chronic skin eruptions. The bracing mountain air found here also enhances the value of a visit to this resort. The application of the waters is chiefly in bathing, and they are conducted from the wells in a continual stream at their natural temperature. There are also electric, steam, and hot-air baths; massage and gymnastics. There are several bath establishments, the property of the Government, two excellent ones, the Great Bathhouse and the König Karls Bad, and others for the poor or those of slender means. There are a variety of outdoor amusements, such as fishing, shooting, and tennis; and, as in almost all these resorts so largely visited by English and Americans, an English church.

Not far from Wildbad is Schömberg (2,130), where is situated a sanatorium for pulmonary tuberculosis conducted by a physician formerly attached to the Falkenstein Sanatorium, and in a similar manner.

St. Blasien (2,530 feet), in the southeastern part of the Black Forest, has an excellent climate and situation, affording mountain and forest air, and is resorted to both in summer and in winter. There are pleasant walks among the forests in the neighborhood, and the paths are arranged for the "Terrain-Cur." There is a well-known sanatorium here, open the whole year for the open-air treatment of pulmonary tuberculosis.

At Nordrach (1,470 feet), near the centre of the Black Forest, is Dr. Walther's celebrated "Nordrach-Colonie," in a protected position, looking toward the south. The success of Dr. Walther in the treatment of pulmonary tuberculosis has given this place and name a world-wide fame; with the English, especially, this sanatorium is very popular, and the sanatoria, now so frequently being established in England, follow Dr. Walther's methods, and sometimes take the name "Nordrach."

The climate at all these open-air resorts in the Black Forest is practically the same: the air is fresh and pure, fairly dry and equable, and although cold in winter (mean winter temperature, 29.6° F.) it is sunny and there is protection from the wind. The mountain influence is also apparent, and the thickly wooded character of the country is not without value.

Badenweiler (1,450 feet), in the lower part of the Black Forest, is a popular summer resort and spa, and possesses great beauty of situation and an excellent climate, pure air with a moderate humidity of seventy per cent., and an equable, mild temperature. The mean average monthly temperatures are: May, 53.8° F.; June, 61.5° F.; July, 64.6° F.; August, 62.8° F.; September, 57.4° F. The yearly rainfall is about forty inches. Badenweiler is used much more for a climatic health resort than for its waters, which are indifferent thermal (of 84° F.). They are used in drinking, in gargling, and in baths; and for various maladies—gout, rheumatism, neurasthenia, chronic neuralgia, diseases of the skin, catarrh of the respiratory passages, dyspepsia, dysmenorrhœa, etc. There are two public bathing pools, the "beautiful marble bath," an open swimming pool, and bath-rooms in a number of hotels. The baths are sometimes artificially heated, and common salt is added to render them more stimulating. The milk and whey cures can also be taken here. The sanitary conditions of the town and the water supply are good. There is also a steam disinfecting apparatus. The accommodations are good in hotels and pensions. In the vicinity of Badenweiler are various resorts of different elevations which offer favorable climatic conditions.

Rippoldsau (1,856 feet) is situated in about the centre of the Black Forest near the Kniebis Mountains, in a thickly wooded valley, with typical forest scenery, rocky precipices, cascades, and the ever-present fir trees. The waters are gaseous chalybeate, containing bicarbonate of iron, sulphate of sodium, carbonate of calcium, and free carbonic acid gas. The waters are taken internally in anæmia, chlorosis, debility, dyspepsia, catarrhal conditions of the stomach and bowels, etc. Iron baths are also used from two springs containing a small amount of iron and rich in carbonic acid gas. Mud baths are used extensively here, and are said to be effective in chronic rheumatic swellings, various sexual disturbances such as amenorrhœa, spermatorrhœa, and impotence. There are also pine-needle, electric, and sand baths, with massage and gymnastics. The baths are heated by Schwarz's method, which consists of the introduction of steam into the double bottoms of the separate baths. There are two well-appointed bathhouses containing twenty-five bathrooms. The yearly average is about thirty-five hundred baths. The water from some of the springs, viz., Josef's, Leopold's, and Wenzel's, are bottled and largely exported, as is also the salt (Rippoldsau salts). The accommodations are good. The rural simplicity of the place remains, many of the old-time costumes being still worn by the natives.

There are numerous other small spas and climatic resorts in the Black Forest, all attractively situated and affording opportunities for hydrotherapeutic treatment or open-air life in a fresh invigorating atmosphere. Indeed, a walking, bicycle, or automobile tour through this most picturesque region may well be considered a cure in itself for tired nerves and the mentally overworked; and the writer, from personal experience, can bear witness to the charm and restfulness of such an excursion: the air is so fresh and invigorating, the food so good, the country scenery so attractive, and the singing of the skylarks so sweet and seductive.

Edward O. Otis.

BRONCHIECTASIS.—Dilatations of the bronchi, either diffuse or circumscribed, are known as bronchiectases. They are of common occurrence and arise from a great variety of causes. In all cases, however, the essential cause of bronchial dilatation is to be found in a weakness of the bronchial wall or an increase of pressure within the bronchus, or in a combination of both these factors. Bronchiectases appear both clinically and pathologically in such a variety of forms that their classification is difficult. According to their etiology they may be divided into the following forms:

Bronchiectasis.
1. Congenital.
2. Atelectatic.
3. Vicarious.
4. Emphysematous.
5. Inflammatory.

GENERAL MORBID ANATOMY.—The condition of bronchial dilatation may be single or multiple, one or more of the bronchi being affected. In some cases the entire bronchial tree is dilated (*universal bronchiectasis*). When but one bronchus is dilated the condition is usually found