autoclave, is shown in Fig. 5068. It consists of a strong boiler, in the bottom of which a small quantity of water is placed. The articles to be steamed are placed in a wire basket, which is set on the bottom of the boiler. The lid is closed with a rubber gasket and securely held in place by thumb screws. Inasmuch as the amount of aqueous vapor in a given space as well as the temperature, in the case of confined steam, is greater than with flowing steam, it follows that the autoclave is considerably more efficient. Thus steam at 130° C., under pressure, will destroy instantaneously spores which would resist flowing steam at 100° for five or six hours.

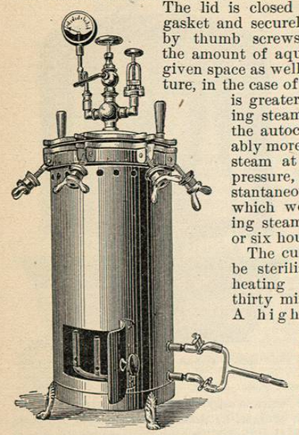

FIG. 5068.—Chamberland Autoclave for Sterilizing by Steam Under Pressure. (Novy.)

The culture media can be sterilized by a single heating for fifteen to thirty minutes at 110° C. A higher temperature should be avoided, as it tends to alter the reaction of the media. Glass apparatus, filters, rubber, etc., can be sterilized by heating at 120° for half an hour. Infected animals can be subjected to 120° for the same length of time, or to 130° or more for a less period.

It must be remembered that the autoclave requires more care than an ordinary sterilizer owing to the danger of explosion. The following points should be observed in its use: Enough water should be present; after the burners are lighted, the steam valve should be left open until all the air has been expelled; when the steam has flowed rapidly for one or two minutes the valve is closed; as soon as the desired temperature is indicated on the gauge, the burners are turned down, so that this temperature is maintained for the required time; the burners are then turned off, but the steam valve is not opened until the temperature has fallen below 100°, after which the lid can be removed. The safety valve should be tested to open at about 125° C. It is a good rule not to leave the autoclave out of sight while the temperature is rising.

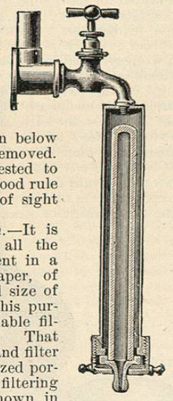

FIG. 5069.—Pasteur-Chamberland Filter.

5. *Sterilization by Filtration.*—It is possible to remove completely all the organisms which may be present in a liquid by filtration. Filter paper, of course, on account of the small size of the bacteria cannot be used for this purpose. There are only two reliable filters for bacteriological work. That known as the Pasteur-Chamberland filter is the best, and is made of unglazed porcelain. The form, as used for filtering water for domestic use, is shown in Fig. 5069. The original French filters are to be preferred to the German imitations. They are made in two grades; that marked *F* is more porous than that marked *B*.

The Kitasato filter, a narrow form of the above, is also made of unglazed porcelain, and is intended for the filtration of very small amounts of liquid. This can, however, be done also with the former. It is shown in Fig. 5070.

The Berkefeld filter (Fig. 5074) consists of closely packed infusorial earth. It can be obtained in several sizes, having the general shape of the Chamberland Pasteur bougie. It is considerably more porous than the porcelain filter, and is therefore adapted for rapid filtration, but it should be borne in mind that it is more likely to allow the passage of bacteria.

FIG. 5070.—Kitasato's Filter.

A useful form of apparatus for holding the Pasteur-Chamberland bougie, that of Martin, is shown in Fig. 5071. It consists of a metal cylinder with a funnel-shaped top, which permits the filtration of the culture through filter paper previous to its passage through the bougie, and thus obviates or lessens the clogging of the latter. A rubber ring serves to make a tight joint when the bougie is held in place by the lower screw cap. The lower end of the bougie is connected with a piece of vacuum rubber tubing to a globe receiver. The entire apparatus is sterilized by heating in an autoclave. The filtration may be carried on by gravity or an aspirator may be connected with the upper tube of the globe receiver. When the filtrate is to be transferred, the drawn out-side tube is scratched near the end with a file, and then broken off, after which the tube is flamed and the liquid is drawn off into sterile tubes or flasks. This globe receiver can be used until the drawn-out tube is too short, when a new tube is fused on.

A better form of a globe receiver is shown in Fig. 5074. This is provided with three side tubes, which are plugged with cotton, after which the receiver is sterilized in a dry-heat oven. When it is to be used, the cotton is removed from the tube *D*, which is then connected with the sterile bougie by means of a piece of sterile vacuum tubing. The horizontal tube *F* is similarly connected with the sterile drawn-out glass tube *G*. The tube *E*, with its cotton plug in place, is connected with a Chapman pump. The filtrate may be withdrawn by means of a sterile bulb pipette, or in the same way as from the receiver of Martin. The advantage lies in the short rubber tubing, compactness, and the convenience in attaching the drawn-out tube. This vacuum receiver can be obtained in several sizes, such as one-quarter, one-half, one, and one and one-half litre capacity.

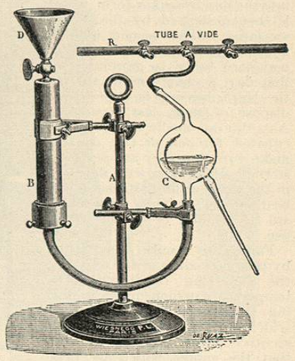

FIG. 5071.—Martin's Filter.

Instead of a metal cylinder to hold the bougie, Novy has devised one of glass. This is shown in Fig. 5072. The necessary tight joint between the bougie and the glass cylinder is made with a rubber ring, and the bougie is brought up tight into place by means of small vises, which act on the flange and on an iron washer. The arrangement is shown in Fig. 5073. If desired the liquid can be filtered under pressure, in which case the rubber stopper at *H* is connected with a tank of compressed air. The cylinders are made to withstand a pressure of over one hundred pounds.

The Berkefeld filter may be attached to the above glass cylinder by means of a rubber and iron washer and the clamps mentioned (Fig. 5074). A more convenient arrangement is to use a cylinder of brass of suitable length and width, threaded at each end. The lower end is provided with a screw cap, through which the metal end of the Berkefeld bougie is passed. The upper end is also closed with a screw cap, provided with a three-eighths of an inch nipple,

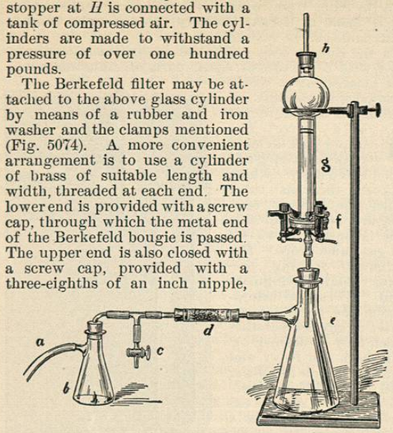

FIG. 5072.—Novy's Filtering Apparatus.

which serves to connect the cylinder with the compressed air. The tip of the Berkefeld is connected with a globe receiver. The filtration can then be carried out by gravity, by aspiration, or by pressure.

The filtration of liquids constitutes an exceedingly important part of bacteriological technique. By its means the soluble products of bacteria may be separated from the solid cells. In this way the toxins of many pathogenic bacteria are prepared. Again it is by the filtration process that it has been possible to demonstrate the existence of the so-called ultra-microscopic organisms. While the common bacteria will not pass through a filter, there are a number of diseases in which the cause is so minute that it will go through the Berkefeld, and at times, even through the Pasteur-Chamberland bougie. Yellow fever, sheep pox, foot-and-mouth disease, contagious pleuro-pneumonia of cattle, chicken pest, rinderpest, horse sickness, molluscum contagiosum of birds, and the "mosaic disease of tobacco" are of this class. So also is that of rabies. The fact that a given filtrate infects is not proof that the cause is always in this extremely minute form. It may be that the real organism is relatively large, as in the case of the rat trypanosome, and yet Berkefeld filtered cultures of this will often infect animals. This is due to the existence of a minute stage in the development of the organism. It is therefore to be expected that the pathogenic protozoa, though they themselves may be large, may give rise to filterable sporozoites.

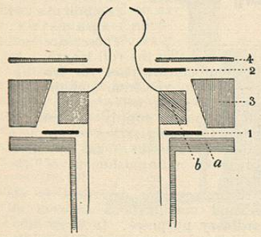

FIG. 5073.—Connections for the Novy Apparatus. 1, 2, 3, Rubber rings; 4, iron washer.

6. *Sterilization by Chemicals.*—This principle is applicable only to a limited extent to nutrient media. The addition of such substances as carbolic acid or mercuric chloride will serve to destroy the organisms which may be present, but since these compounds cannot be removed from the medium, it follows that it cannot be used for culture purposes. A few substances have, however, been used with this object in view. Thus if chloroform is added to milk or blood serum, and is allowed to act long enough, it will bring about sterilization. The remaining chloroform can finally be driven off by means of gentle heat and by aëration. Ether has been used in the same way, and indeed this is a useful procedure for sterilizing such weak cultures as those of cholera.

Glycerin, as is well known, is added to vaccine with the object of destroying the common pus-producing organisms which are so often present. It certainly will in time destroy all of these accidental bacteria, but unfortunately prolonged exposure of the vaccine virus to the glycerin damages it as well.

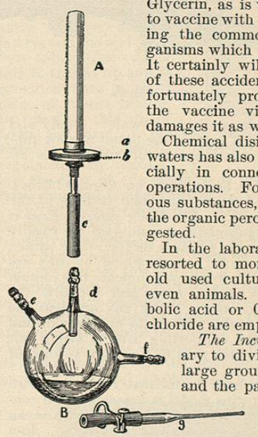

FIG. 5074.—Berkefeld Filter showing Manner of Attachment. Globe receiver. (Novy.)

Chemical disinfection of drinking-waters has also been proposed, especially in connection with military operations. For this purpose various substances, such as bromine and the organic peroxides, have been suggested.

In the laboratory this method is resorted to more or less to sterilize old used cultures, test tubes, and even animals. Five-per-cent. carbolic acid or 0.1-per-cent. mercuric chloride are employed.

The Incubator.—It is customary to divide bacteria into two large groups—the saprophytic and the parasitic—according as to whether they grow in nature on dead matter or in the living body. Among the latter are classed the disease-producing bacteria. In general the optimum temperature for the growth of the saprophytic organisms is about 25° C., whereas the pathogenic bacteria thrive best at the temperature of the body. In order to supply this requirement it is necessary to use an incubator or thermostat, the temperature of which can be maintained without variation at any desired level. Various forms of apparatus have been devised for this purpose; that of Koch is shown in Fig. 5075. It consists of a double-walled box of copper, the sides and top

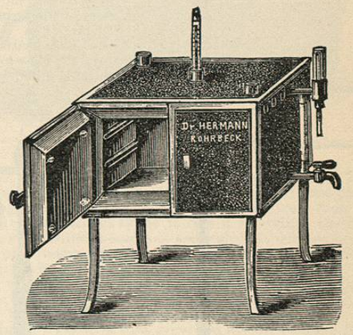

FIG. 5075.—Koch's Incubator.

being covered with felt. The space between the walls is filled with water. In the top is an opening communicating with the interior air space, and in it a thermometer is placed to indicate the temperature. The openings in the corners communicate with the water space. One of these is intended to hold a thermo-regulator, while

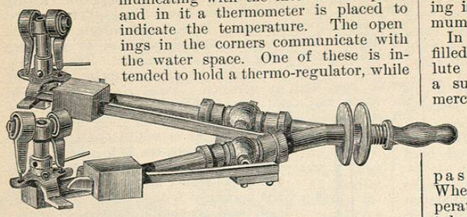

FIG. 5076.—Koch's Safety Burners.

the other serves for the addition of water. Inner and outer doors are provided, and in the better models provision is made for ventilation and for keeping the air moist.

The apparatus may be heated with an Argand burner. The ordinary Bunsen burner is not used because of the danger of "shooting back." The Koch safety burner is to be preferred, for it automatically shuts off the supply in case the gas should by any chance happen to be turned off. It consists of two iron spirals which, as they are heated, expand, and in so doing communicate this motion to an arm which then swings under and supports the weighted lever of the valve. If by any accident the flame should become extinguished, the spirals cool and contract; this causes the supporting arm to swing out from under the lever, which then falls and thus shuts off the gas (see Fig. 5076).

In case gas is not available the incubator is heated with an oil lamp. The Sartorius model is especially well constructed for this form of heating. A good substitute can always be found in the ordinary egg incubator. In a few places the heat is supplied by means of electric hot plates.

By far the most important accessory to an incubator is a thermo-regulator, which will automatically control the supply of gas and hence the temperature of the oven. Several of the more common forms are shown in Fig. 5077. The Reichert form, though very widely used, is far from being the most satisfactory. The lower bulb is filled with mercury, which as the temperature rises shuts off the opening through which the gas enters. In order to prevent the flame from being extinguished a minute opening is made in the gas delivery tube whereby a minimum flame can be maintained.

In the Bunsen form the lower compartment is nearly filled with a mixture of equal parts of ether and absolute alcohol, after which a sufficient quantity of mercury is added to act as a valve. The upper part is closed with a stopper, through which passes the gas tube. When the proper temperature is reached, this tube is pushed down till the gas flame drops. The minimum opening prevents total extinguishment. By careful manipulation the regulator can be set at any temperature which may be desired.

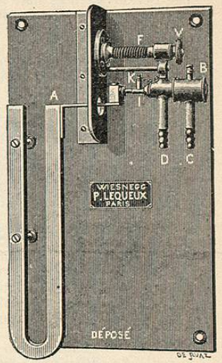

FIG. 5078.—Roux Metallic Thermo-Regulator.

In both the Novy and Dunham forms the lower bulb is filled with absolute alcohol. As this expands it acts against a column of mercury, which in turn shuts off the supply of gas. The lateral screw permits the adjustment of the regulator to the desired temperature. In the former the minimum supply can be regulated to a nicety. This enables it to be used for a water-bath, or for a small or large incubator. It can be obtained with the alcohol cylinder of different sizes, according to the use for which the apparatus is intended.

The metallic regulator of Roux is intended for controlling the temperature of large water-baths and of incubator rooms, for which purpose no better device can be found. It is made in the two forms, the straight and the **U** shape, shown in Fig. 5078. It consists of two metal bands having different coefficients of expansion. These are soldered together the full length. As the temperature rises, the free upper arm moves from and thus releases a spring valve, which shuts off the main supply of gas. A minute opening serves to supply a minimum amount of gas, and thus prevents extinguishment of the flame.

The incubator described in its several modifications answers all ordinary purposes. In large laboratories they can, however, be dispensed with almost entirely, and their place is taken by the incubator room. By this is meant a room, usually about eight feet cube, which is maintained at a constant temperature. This arrangement

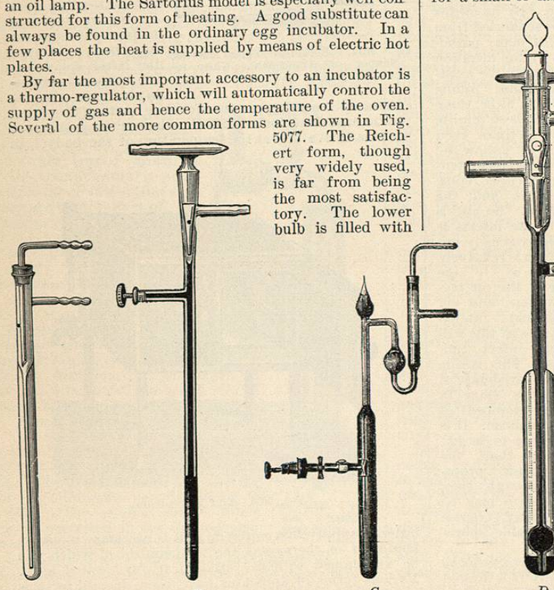

FIG. 5077.—Thermo-Regulators. *A*, Bunsen's; *B*, Reichert's; *C*, Dunham's; *D*, Novy's.

was first employed at the Pasteur Institute, where the heat is conveyed to the room by means of large pipes along the wall, filled with water, or better with glycerin. The circulating liquid is heated by a small gas stove placed on the outside of the room.

A more simple and thoroughly efficient procedure is to place a small gas stove in the centre of the room. This stove, which can be obtained of the French dealers, is so constructed that the gases of combustion are carried out of the room into a flue.

Another procedure of limited application is to heat the room with steam coils. The regulation of the heat in this case is accomplished by means of an automatic steam valve operated by compressed air.

The construction of the room requires no special care. The walls, whether of brick or of plaster, should be given several coats of white zinc. Shelves, water, gas and electric light, and a window should be provided.

The regulation of the temperature in the rooms heated by gas is done by means of the Roux **U**-shaped regulator. All the connections should be of metal to lessen the chances of fire. In order to have a temperature record it is advisable to place in the room a thermometrograph, the best form of which is made by the Richard Frères, of Paris.

Gas-Pressure Regulator.—The best results with any form of thermo-regulator are obtained when the gas pressure is constant, or nearly so. When the variation is considerable it is advisable to pass the gas through a pressure regulator before it reaches the thermo-regulator. There are several forms of apparatus for this purpose. The Moitessier regulator is shown in Fig. 5079. It consists of a cylinder *A*, which is filled to the level of *G*, with a mixture of equal parts of glycerin and water. On this is floated the metal shell *B*. The gas is admitted to the interior of *B*, through the tube *K*, the pressure being indicated by the manometer *P*. The gas flows into *B* until it is filled, when it raises it up and shuts off the supply of gas by closing the valve *D*. The pressure on the burner is regulated by the weights placed in the pan *H*, which is connected with *B* by the rod *G*. The amount of pressure on the burner is indicated by the manometer on the left of the apparatus. The burner is connected with the apparatus by means of a rubber tube attached to *I*, and the height of the flame is regulated by the stopcock *M*.

A cheaper and more simple regulator is that devised in Novy's laboratory by Murrill and shown in Fig. 5080. The gas passes into a cylinder which floats in liquid petroleum and leaves by two tubes at the bottom, one of which is connected with the thermo-regulator, the other with a manometer. The cylinder is weighted so as to give the desired pressure to the outflowing gas.

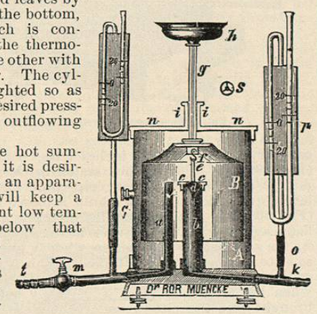

FIG. 5079.—Moitessier's Gas-Pressure Regulator.

During the hot summer months it is desirable to have an apparatus which will keep a fairly constant low temperature, below that which would cause the gelatin cultures to melt. There are incubators constructed for this purpose which furnish a supply of ice-cold water when the temperature rises above a given point. If the temperature drops too low an electric lamp is turned on.

When the temperature of the water as it leaves the ground is about 15° it is possible to use the simple apparatus shown in Fig. 5081. This is made of galvanized iron. The inner box is held in place by means of a couple of stout rods. The water enters at the bottom through the small tube, which stops short on the inside of the outer box. The water then flows under and around the inner box, and eventually reaches at the farther end the wide outflow tube. The end of this is turned up and is provided with a short piece of rubber tubing. By moving this up or down the level of the water in the box can be regulated. By regulating the flow of the water it is possible to maintain a fairly constant temperature in the inner compartment.

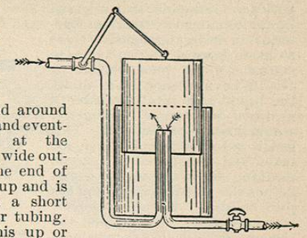

FIG. 5080.—Murrill's Gas Pressure Regulator. Cross-section. (Novy.)

The Methods of Cultivation.—The fundamental basis of bacteriology may be said to be the fact that it is possible to cultivate artificially, and that in pure condition, nearly all of the known forms of bacteria. Until methods had been devised for this purpose it was not possible to determine definitely the part played by any organism either in the ordinary phenomena of fermentation or in the more mysterious processes of disease. To arrive at a demonstration of the causal relation of a given organism to the change which it is supposed to induce it is necessary to do two things: First, the organism must be isolated in pure culture; and second, the pure culture, once obtained, must be maintained by transplantation. The pure culture thus kept up through a series of transplantations, or generations as they are called, can then be tested upon animals to see if it will reproduce the disease; upon suitable media to ascertain if it will cause the kind of fermentation, the pigment, or the light which it is supposed to produce.

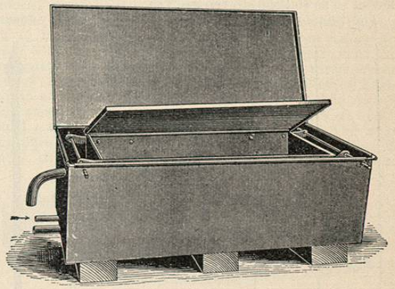

FIG. 5081.—Novy's Low-Temperature Incubator.

By a pure culture is meant one which is derived from a single cell. A given bacterium, small as it is, multiplies by division, and thus gives rise to two new individuals. These in turn grow and divide, yielding four cells. This process is kept up till many millions of organisms constitute the offspring of a single cell. Inasmuch as the division of bacteria is very rapid under favorable conditions, many dividing every half-hour, it follows that in a few hours a visible growth may be seen where at the beginning but a single cell was present. If the medium in which they are growing is liquid, it will usually become cloudy because of the disseminated bacteria.

The early methods of cultivation, as employed by Pasteur, made use of liquid media. Under these conditions it was exceedingly difficult to obtain pure cultures, and indeed it was largely a matter of chance and patience. Suppose that a given liquid contains two kinds of bacteria; in order to separate these so as to have a single cell as a starter for the pure culture it would be necessary so to dilute the liquid that in all probability a drop, or a cubic centimetre, would contain but one cell. This small quantity would then be taken and transferred to a sterile medium, and in this way it would be possible to obtain presumably pure cultures. Failure, however, was necessarily frequent and the element of doubt always remained.

The introduction of the nutrient gelatin by Koch made it possible to secure pure cultures with the greatest of ease. All that was necessary was to inoculate the liquefied gelatin with the mixture of bacteria, and after thorough agitation so as to separate each cell from its neighbor, to pour the liquid on to the surface of a sterile plate. The gelatin now solidifies and imprisons, as it were, the separated cells. Each of these now multiplies and reproduces its kind; eventually, in the course of a day or two, a small growth, perhaps of the size of a small pinhead, appears. This is called a colony, and since it is derived from a single cell it constitutes a pure culture. Such is the principle of the dilution method for obtaining pure cultures. The isolation once accomplished, all that is necessary is to transplant the colony to sterile culture media so as to keep up the growth.

The transferring of bacteria is usually done by means of a platinum wire. The wire, which should be about two inches long and fairly stiff, about No. 21 in size, is fused into the end of a glass rod. According to the object in view it is either straight, bent, or is provided with a loop as shown in Fig. 5082.

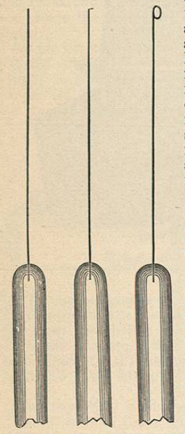

Fig. 5082.—Platinum Wires, Fused in Glass Rods.

At times a bunch of very fine platinum wires attached to a holder, the so-called Kruse's brush, is used to spread the material over the surface of the media. A glass rod bent at right angles is also very useful for this purpose. The Roux spatula of nickeled steel was first employed for the purpose of transplanting bits of diphtheritic membrane to the culture tube. A similar spatula made of thick iron wire is extremely useful for transferring moulds and compact growths, such as that of actinomyces. The Nuttall platinum spear is particularly useful for transferring bits of tissue, blood, etc., to the nutrient media. These two instruments are shown in Fig. 5083.

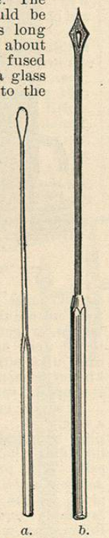

Fig. 5083.—*a*, Roux spatula; *b*, Nuttall's platinum spear.

The transferring of liquids, in large or in small quantities, can best be done by means of drawn-out glass tube pipettes, as is practised by the Pasteur School. This technique is at once simple and invaluable. The preparation of these pipettes will be understood from Fig. 5084.

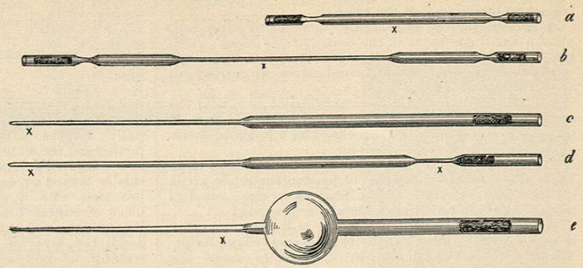

Fig. 5084.—Pasteur Pipettes, showing Method of Preparation. (Novy.)

The glass tubing, which has a diameter of about 8 mm., is cut up into lengths of about twelve inches. By means of the blast lamp a slight constriction is made at about two inches from each end. This serves to prevent the cotton plug from falling down, and also tends to keep the liquid from reaching the cotton. The ends of each tube are then carefully rounded out in the flame. A piece of cotton is then pushed into the end of each tube. The tubes thus prepared (Fig. 5084, *a*) are then sterilized in the dry-heat oven, after which they may be stowed away for future use.

Whenever it is desired to make a pipette, one of these tubes is heated in the middle in a blast flame, and when the glass has thoroughly softened, the two halves are slowly drawn apart. A relatively wide, thick-walled capillary, about sixteen inches long, is thus obtained (Fig. 5084, *b*). This is then sealed in the flame in the middle, and the result are two pipettes. For transferring larger quantities of liquid a bulb is blown in the pipette (Fig. 5084, *e*). This is made by directing a narrow blast flame against the tube, which is at the same time rotated. As the glass softens the ends are slightly pushed together, so as to form a thick ring of glass. This is repeated once or twice. Finally a large blast flame is turned on, and when the thickened glass is perfectly soft, the end is brought into the mouth and the bulb is blown. The glass should be rotated during this operation, and in fact in all work of this kind.

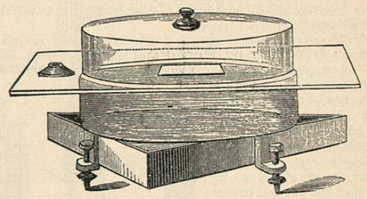

Fig. 5085.—Koch's Levelling Apparatus.

To use a pipette, the mouth end should first be rolled for a few seconds in a flame so as to insure sterility; the capillary end is then scratched with a file and the tip is broken off, after which the capillary is flamed. As soon as the tube has cooled, which fact can be ascertained by blowing through the pipette against the back of the hand, it is ready for use. The closure of the pipette when it is filled with the liquid is effected by means of the tongue.

The great value of the pipette lies in the fact that it can be made in a few minutes, and can be used to transfer liquids from one tube to another, for drawing blood from the heart, fluids from the cavities, etc. It is indeed even more useful than a platinum wire.

Plate Cultures.—Solid media, such as gelatin or agar, either plain or modified, are employed for this purpose. The starting-point in this method were the slide cultures used by Koch in his early investigations. The liquefied gelatin was poured upon the surface of sterile glass slides, which were levelled and kept cool by means of the apparatus shown in Fig. 5085. The lower dish was filled with ice water and the whole was set true by the aid of a small spirit level. A series of parallel streaks was then made on the solid gelatin by means of a platinum wire, which was dipped in the material to be planted. A number of slides were thus made, after which they were stacked on glass benches (Fig. 5086), and placed in a moist chamber to develop (Fig. 5087). The first streak, on account of the large number of organisms planted, would probably yield a continuous solid growth. The next streak would have fewer germs, and the succeeding ones still less until eventually only single germs would be deposited, separated by an appreciable distance from the following ones. Wherever a single organism was deposited, as a result of multiplication, a colony would soon make its appearance. (See Plate LXI.)

The slide method was soon improved by substituting larger glass plates (10 × 13 cm.). Instead of making streak dilutions as just given, the gelatin was liquefied, inoculated, and poured out upon the sterile plates, which were cooled on the plating apparatus. This method of plating may be used when the special Petri dishes, ordinarily employed, are not obtainable. The fact that the method required a lot of apparatus, slides, slide-box, levelling apparatus, ice, moist chamber, etc., as well as the fact that contamination from the air and from the dripping of the superposed plates was unavoidable, led Petri to introduce the modification which has almost entirely supplanted the older method.

Fig. 5086.—Glass Benches and Culture Slides.

Gelatin Petri Plates.—In this method, as in the preceding, the gelatin is first melted by immersion in warm water for a few minutes. By means of a sterile, looped, platinum wire a small quantity of the material to be examined is transferred to a tube of liquefied gelatin, marked 1. By means of the wire the material is thoroughly mixed with the gelatin. Another tube, marked 2, is then placed beside the first, from which three loopsful of gelatin are carried over to tube 2, with the contents of which they are well mixed (Fig. 5088). A third tube, marked 3, is then placed beside number 2, and three loopsful of gelatin are transferred from tube 2 to tube 3. It is evident from this procedure that even if the first tube received a million germs the second tube would contain only a small fraction, and the third tube would contain still less. The platinum wire must of course be sterilized whenever an inoculation is made into a new tube.

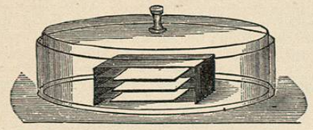

Fig. 5087.—Moist Chamber with Stacked Plates.

A number of Petri dishes (Fig. 5089), which are 10 cm. in diameter and 1 cm. high, are previously sterilized by heating in a dry-heat oven for one hour at 150°, or for a few minutes at 200°, and allowed to cool. To pour the plate, the cotton is removed from one of the tubes, and the open end is rolled for a few seconds in the flame so as to sterilize it. In a few seconds the end of the tube becomes cool, after which the contents are poured out into the Petri dish. The lid of the latter is removed just sufficiently to allow the gelatin to be introduced. By tilting the gelatin is then spread all over the bottom of the dish. The latter is then set aside in a cool place for the gelatin to set. With a good gelatin this will take place even in the ordinary room within a few minutes. The remaining gelatin tubes are poured in the same manner. Each plate should be numbered to correspond to the tube from which it was made. They should be marked also with the date and the kind of material used. A Faber's colored wax pencil is used for this purpose.

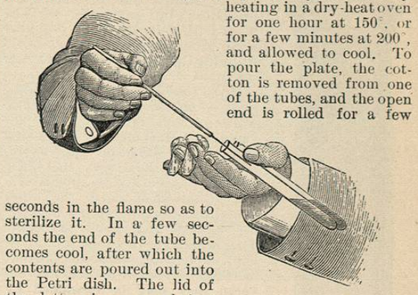

Fig. 5088.—Method of Holding the Tubes when Making Dilutions.

Agar Petri Plates.—Inasmuch as gelatin melts at about 25° C. it follows that the method just given cannot be used when the organism requires the temperature of the incubator. In such cases it is necessary to resort to the use of agar. The nutrient again is first melted by heating in a water-bath at 100° C. The flame is then turned out and the tubes are allowed to cool in the water bath until a temperature of about 45° is reached. The agar solidifies at about 40°, and consequently the dilution must be made rapidly and the plates poured before the point is reached. Dilution cultures are made in the same way as just given for gelatin. The three agar tubes are then poured out into the corresponding sterile Petri dishes. The agar promptly solidifies, and for that reason the spreading of the agar over the bottom must be hastened. The agar plates are then set aside to develop either at the temperature of the room or at that of the incubator.

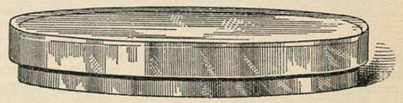

Fig. 5089.—Petri Dish for Plating.

Esmarch Roll-Tube Culture.—This modification of the plate method does away with the use of any special container other than the test tube. The dilutions in gelatin are made in the usual way. According to the original directions the cotton plug was cut off short, and the end of the tube was covered with a close-fitting rubber cap. The tube was then immersed and rotated in an almost horizontal position in ice water. The gelatin solidified in an even film over the inside of the test tube (Fig. 5090).

A more convenient way of rolling the tubes was de-

vised by Booker. With the aid of a large test tube filled with warm water a groove is melted into a block of ice. The gelatin tubes are then rolled in this groove until the gelatin solidifies in a smooth, even film. Nuttall has modified this procedure by replacing the ice block with a marble block provided with grooves for the test tubes. Running tap water serves to cool the tubes.

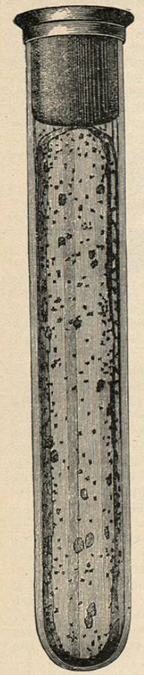

FIG. 5090.—Esmarch's Roll Culture.

If the tubes are not rolled smoothly they can be softened by gentle warming and rerolled. One advantage of this method lies in the fact that desiccation can be retarded more than with the other methods. Air contamination is likewise diminished. On the other hand, the presence of a few liquefying bacteria may spoil the tube. The Esmarch roll tubes should be kept in a cool place to prevent melting. When the colonies develop they may be examined by placing the tube on the stage of the microscope. To transplant the colonies a platinum wire, provided with a hook, as shown in Fig. 5082, should be used.

Shake Cultures. — Dilutions are made in gelatin or agar as heretofore described. The tubes are then solidified in an upright position and allowed to develop. If it is desired to transplant a given colony the test tube should be scratched with a diamond at about the level of the colony. On touching the scratch with a hot rod, the crack can be led around the tube, after which the two parts can be separated. By means of a sterile knife the medium can be cut and the colony exposed. In the case of agar the entire cylinder of agar can be forced out of the tube into a sterile dish by the cautious application of a flame to the lower end of the tube.

The method offers a convenient means of determining whether or not the organism planted generates gas. If such is the case gas bubbles will make their appearance in the medium. As will be seen later this method is also useful in connection with the cultivation of anaerobic bacteria.

Streak Cultures.—This procedure, which is essentially the same as that used by Koch in his slide cultures, is very frequently made use of at the present time. Thus, sterile gelatin or agar may be poured into sterile Petri dishes, and after the material has solidified a series of parallel streaks may be made with an infected wire. The Kruse platinum brush may be used to spread the organisms over the surface. A narrow glass rod, bent and flattened at the end, has been used for spreading gonorrhœal pus over plates. Cotton swabs are used for the same purpose in the case of diphtheria.

As in the case of the Esmarch roll tube, the Petri dish may be omitted in this method. In that event the gelatin or agar is melted and allowed to solidify in an inclined position. The streaks are then made on the surface of the inclined medium. Potato tubes are inoculated in the same way. (See Plates LX. and LXI.)

To obtain perfectly isolated colonies by this method the same wire should be used to make parallel streaks on each of four or five tubes. When the colonies develop transplantations can be made by means of a bent wire.

Hanging-drop Cultures.—A concave or well slide, shown in Figs. 5105 and 5106, is used. The cover glass must first be sterilized by passing it several times through a flame. A large drop of sterile bouillon is then placed in the centre, and this is then inoculated with the germ to be studied. The slide with a ring of vaseline is then inverted and brought down upon the cover-glass, after which the preparation is turned over. Care must be taken to see that the vaseline closure is perfect. This method of cultivation is used to study the multiplication of the bacteria under the microscope.

Hanging-block Cultures.—In order to be able to study the morphology and the multiplication of the diphtheria bacillus to better advantage than that afforded by the hanging drop, Hill devised the following procedure: Melted nutrient agar is poured into a Petri dish to a depth of about one-eighth to one-quarter inch. When cool, a block of agar is cut out, about one-quarter to one-third inch square, and of the thickness of the agar layer in the dish. The block is placed, under surface down, on a slide and protected from dust. A suspension of the growth to be examined is then made in sterile water or a bouillon culture is used. The suspension is spread over the upper surface of the block as if making an ordinary cover-slip preparation. The slide and block are then set aside in the incubator at 37° for five or ten minutes, to dry slightly. A clean sterile cover slip is then placed on the inoculated surface of the block in close contact, avoiding large air bubbles. The slide is then removed from the under surface of the block, and the cover slip is inverted so that the agar block is uppermost. With the aid of a platinum wire a drop or two of melted agar is run along each side of the agar block, to fill the angles between the sides of the block and the cover slip. This seal hardens at once and prevents slipping of the block. The preparation is again placed in the incubator for five or ten minutes to dry the agar seal. Finally the preparation is inverted over a moist chamber or suitable well slide. The cover slip is sealed in place with white wax or paraffin. Vaseline cannot be used because it softens at 37°. The "hanging block," thus prepared, is examined on a warm stage or in the incubator room.

Transplantation of Colonies.—The entire object of making plate culture by any one of the several methods given is to obtain single isolated colonies, which can be transplanted to other media and the organism present can then be studied in perfectly pure condition. The colony as indicated is presumably derived from a single cell, and consequently is a pure culture. The transplantations or sub-cultures can be made to gelatin, agar, serum, bouillon, milk, etc. When the colonies are on a plate or in a Petri dish a straight wire is used. The plate is first carefully examined under the microscope, and a colony is selected which is clearly single. If possible it should be the only one in the field of the No. 3, or one-third inch objective. The farther apart that the colonies are the less likely are they to intermingle. As originally directed, the colonies were touched under the microscope by a sterile platinum wire, and in this way a few of the bacteria were removed. Care had to be taken that the wire did not touch the objective or any other part of the gelatin. This operation of "fishing," as it was called, obviously required considerable practice and care. An equally good procedure is to pick out the desired colony under the microscope. The tube of the microscope is then raised, and the point of the sterile wire is brought down so that it cuts the colony and nothing else. The tube is then again lowered and the site of the colony examined to make sure that nothing but the one colony was touched. If such is the case the wire is then used for the sub-culture.

Stab Cultures.—A tube of solid gelatin is taken, the cotton plug is seized by the right little finger and removed. The mouth of the tube is then flamed, after which the wire, laden with the bacteria, is inserted and carefully passed down the centre of the gelatin. The organisms are thus planted along the line of inoculation. The cotton plug is replaced and the tube is labelled and set aside. The form of the growth is then noted from day to day, also the presence of gas, liquefaction, pig-

TEST-TUBE CULTURES.

Reproduced from Huber & Becker's "Untersuchungs-Methoden."

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Fig. 5080.—Esmarch's Roll Culture.

If the tubes are not rolled smoothly they can be softened by gentle warming and rerolled. One advantage of this method lies in the fact that desiccation can be retarded more than with the other methods. Air contamination is likewise diminished. On the other hand, the presence of a few liquefying bacteria may spoil the tube. The Esmarch roll tubes should be kept in a cool place to prevent melting. When the colonies develop they may be examined by placing the tube on the stage of the microscope. To transplant the colonies a platinum wire, provided with a hook, as shown in Fig. 5082, should be used.

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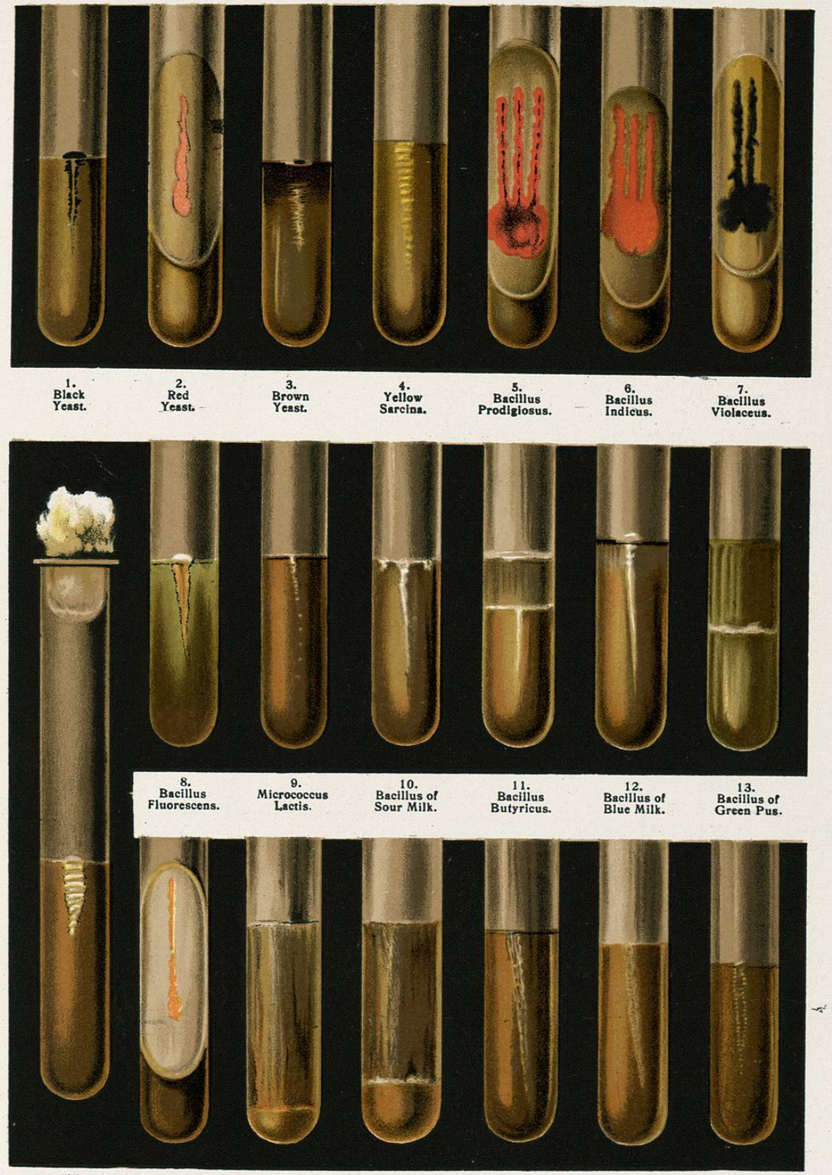

1. Black Yeast.
2. Red Yeast.
3. Brown Yeast.
4. Yellow Sarcina.
5. Bacillus Prodigiosus.
6. Bacillus Indicus.
7. Bacillus Violaceus.
8. Bacillus Fluorescens.
9. Micrococcus Lactis.
10. Bacillus of Sour Milk.
11. Bacillus Butyricus.
12. Bacillus of Blue Milk.
13. Bacillus of Green Pus.
14. Micrococcus Tetragenus.
15. Staphylococcus of Osteomyelitis.
16. The same in gelatine.
17. Staphylococcus pyogenes albus.
18. Streptococcus pyogenes.
19. Streptococcus of erysipelas.
20. Streptococcus of Puerperal Fever.

TEST-TUBE CULTURES.

Reproduced from Huber & Becker's "Untersuchungs-Methoden."

ment, etc. The characteristics of the stab cultures are of the very greatest importance in the identification of bacteria. If the gelatin is old and partially dried, the passage of the needle is likely to cause a split in the medium. This can be avoided by melting and resolidifying the gelatin. (See Plates LX. and LXI.)

Streak Cultures.—These are also known as "smear cultures." The gelatin or agar tubes are melted and solidified in an inclined position. Similarly solidified blood serum is also used; so also are the potato tubes. The infected platinum wire is drawn along the middle of the surface of the medium by making one single streak. The growth develops along the line of inoculation, and spreads in a more or less characteristic manner. (See Plates LX. and LXI.)

Liquid Cultures.—The tubes of sterile bouillon, milk, serum, etc., are inoculated by simply introducing some of the material from a colony by means of the sterile wire.

The sub-cultures from tube to tube are made in the same way as just given. The drawn-out glass tube pipettes and spatulas can be used to transfer the material from one tube to another, or to flasks.

Anaërobic Cultivation of Bacteria.—The methods just given are essentially aërobic, since there is free access of air. As is well known there are two classes of bacteria with reference to their oxygen requirements. The aërobic bacteria live in the presence of air, while the anaërobic thrive only in the absence of oxygen. In order to cultivate the latter special methods must, therefore, be employed which will supply the needed conditions. Numerous procedures have been devised for this purpose, and to give all of these would be beyond the scope of this article. It will be sufficient to indicate the principles which serve as a basis for these methods, and to describe those which are most widely used.

Fig. 5091.—Liborius Deep Stab Culture, showing Growth of the Tetanus Bacillus.

1. *Exclusion of Oxygen.*—This was accomplished by Pasteur in his early work by pouring a layer of oil upon the culture fluid. This served to exclude the air and allowed the bacteria to develop. Koch obtained anaërobic conditions by covering the surface of the gelatin plates with a thin sheet of mica. Others have done the same with glass plates.

The Liborius method of cultivation in deep layers falls under this head. It is simple and is constantly used. Ordinary stab cultures are made in the suitable media, preferably glucose agar. Another tube of agar is liquefied, cooled to about 50°, and the contents of this are then poured on top of the stab culture. Care must be taken to flame the mouths of both tubes so as to avoid contamination. The upper layer of agar serves to keep out the air. The cultures can be prepared equally well by employing agar or gelatin tubes filled with the medium to a depth of about two inches. After the stab is made the line of puncture closes up itself, and the growth then develops in the lower part of the tube, as shown in Fig. 5091.

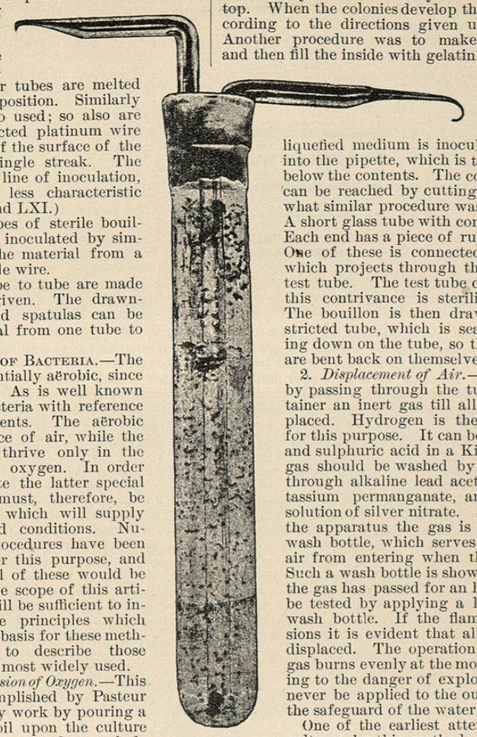

Fig. 5092.—Fraenkel's Modification of Liborius Tube for Anaërobes.

Isolated colonies can also be obtained by this method. The liquefied medium is inoculated and dilutions are made as for shake cultures. The tubes are then solidified, and if necessary an additional layer of medium is poured on top. When the colonies develop they can be reached according to the directions given under shake cultures. Another procedure was to make Esmarch roll tubes and then fill the inside with gelatin or agar.

The drawn-out glass tube pipettes (Fig. 5084) have been used by Roux for this same purpose. The liquefied medium is inoculated and drawn up into the pipette, which is then sealed above and below the contents. The colonies which develop can be reached by cutting the glass. A somewhat similar procedure was devised by Wright. A short glass tube with constricted ends is used. Each end has a piece of rubber tubing attached. One of these is connected with a glass tube, which projects through the cotton plug of the test tube. The test tube contains bouillon, and this contrivance is sterilized and inoculated. The bouillon is then drawn up into the constricted tube, which is sealed by simply pushing down on the tube, so that both rubber ends are bent back on themselves.

2. *Displacement of Air.*—This is accomplished by passing through the tube or a suitable container an inert gas till all the air has been displaced. Hydrogen is the least injurious gas for this purpose. It can be generated from zinc and sulphuric acid in a Kipp's generator. The gas should be washed by passing successively through alkaline lead acetate, six per cent. potassium permanganate, and finally through a solution of silver nitrate. After passing through the apparatus the gas is sent through a small wash bottle, which serves as a valve to prevent air from entering when the current slows up. Such a wash bottle is shown in Fig. 5093. After the gas has passed for an hour or more it should be tested by applying a light as it leaves the wash bottle. If the flame burns with explosions it is evident that all the air has not been displaced. The operation is continued until the gas burns evenly at the mouth of the tube. Owing to the danger of explosion the light should never be applied to the outflowing gas without the safeguard of the water valve.

One of the earliest attempts at making tube cultures by this method was that of Liborius. He made use of a special test tube with a delivery tube fused into the side. After inoculation of the liquid medium gas was passed through, and finally the neck of the test tube, as well as the end of the delivery tube, were sealed in the flame. This method is of only very limited application, and requires much time and is expensive. Fraenkel's modification is a distinct improvement. Ordinary large test tubes are used. These are provided with rubber stoppers and delivery tubes, as shown in Fig. 5092. After the inoculation of the medium and expulsion of the air, the tubes are sealed in the flame. If it is desired to obtain colonies, the tube can be converted into an Esmarch roll tube, as shown in Fig. 5092.

This principle has been adapted in various ways for the purpose of obtaining plate cultures. Kitasato employed a flat bottle, having a tube fused at the lower end. The dilutions were made in the ordinary tubes, after which the material was poured into these flasks, which were connected in series and hydrogen passed through. Finally the ends were sealed by fusing in the flame, while the neck of each flask was closed with a clamped rubber tube. Several modifications of this bottle have been made, but they are little used, since methods