were soon perfected whereby it was possible to make Petri plates in hydrogen.

One of the earliest attempts in this direction was that of Blücher, who made use of a funnel which was

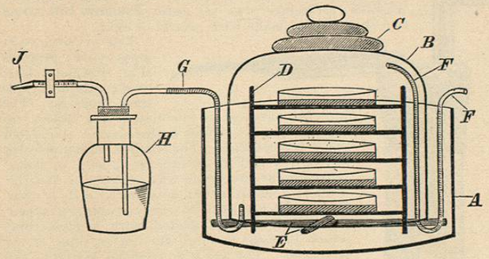


FIG. 5093.—Botkin's Apparatus for Plate Culture of Anaërobes.

weighted with lead and inverted over the plates in a larger dish. Air was excluded by means of glycerin water. Hesse inverted a glass vessel in a circular trough filled with mercury. Liborius used a copper bell jar which was compressed against a rubber gasket by means of set screws; others made use of bell jars inverted upon a ground glass surface. In many respects the Botkin apparatus is useful. It is shown in Fig. 5093. It consists of a metal rack on which are placed the Petri dishes. This is set in a large outer dish which contains about an inch of liquid petroleum. A bell jar is inverted over the stand. The inflow and outflow tubes are of rubber stiffened by a copper wire on the inside. After the hydrogen has been passed for a sufficient length of time, the tubes are withdrawn and the apparatus is then set aside.

The Novy apparatus shown in Fig. 5094 leaves little to be desired. The hollow stopper has two perforations, one of which is connected with a glass tube which extends almost to the bottom of the bottle. In the case of the plate apparatus the tube may be continued by means of a piece of rubber tubing. A perfect seal is obtained by simply turning the stopper through an angle of 90°.

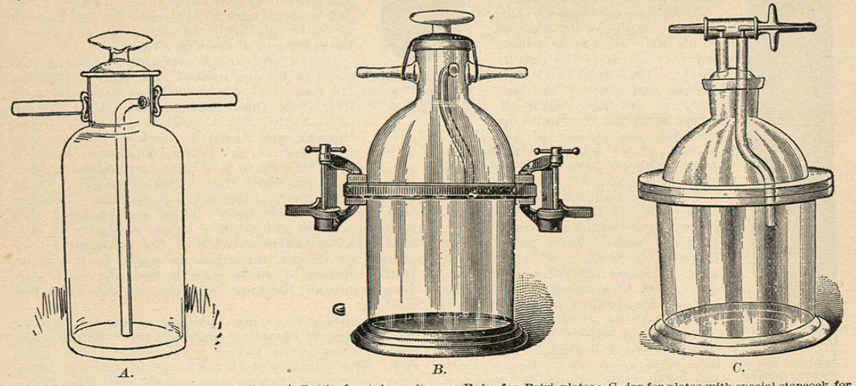


FIG. 5094.—Novy Apparatus for Anaërobes. *A*, Bottle for tube cultures; *B*, jar for Petri plates; *C*, jar for plates with special stopcock for vacuum culture.

The bottle (Fig. 5094, *A*) is made in two sizes, 8 × 16 and 10 × 20 cm., which dimensions do not include the neck. A piece of cotton should be placed on the bottom.

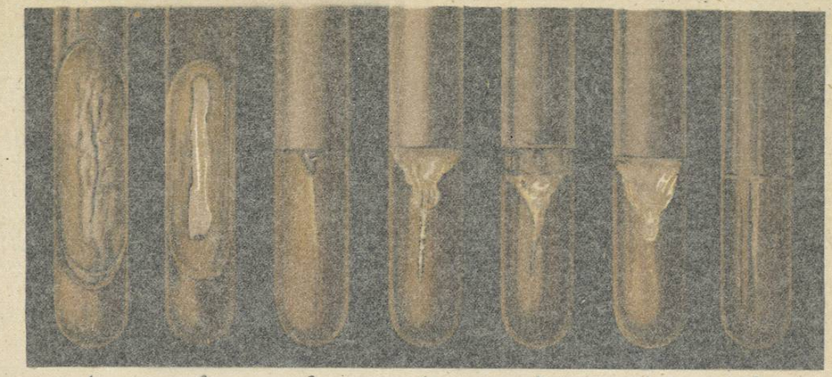
The ordinary test tubes containing any medium are inoculated in the usual way. The cotton plug is then cut off square, and by means of a pair of crucible tongs the tube is lowered into the bottle. It is advisable, if the cotton plug is very tight, to loosen it up by partially pulling it out. A single jar can be filled in this way with a large number of tubes containing either solid or liquid media. The stopper is then put in place and the apparatus connected with a hydrogen generator. When the gas has passed for a sufficient length of time the bottle is closed by giving the stopper a turn. As will be seen this jar can be used likewise for the pyrogallate method.

The plate apparatus shown in Fig. 5094, *B*, consists of two parts. The inner dimensions of the lower part are 12 × 12 cm. The Petri plates are stacked into this compartment. The flanges are covered with a mixture of beeswax and olive oil (1:4). The two parts are then brought together and a rubber band is slipped over the outer edge of the flanges. Two or three clamps or small vises are now applied. The jaws of these should be covered with a piece of rubber tubing. Gas is passed as in case of the bottle, and at the conclusion of this operation the stopcock is given a turn so as to seal the apparatus.

The other modification (Fig. 5094, *C*) has a special stopper, which enables it to be used for vacuum cultures. It can, however, be employed equally well for hydrogen cultivation. Moreover, both forms of the plate apparatus, as well as the bottle, can be used for the pyrogallate method.

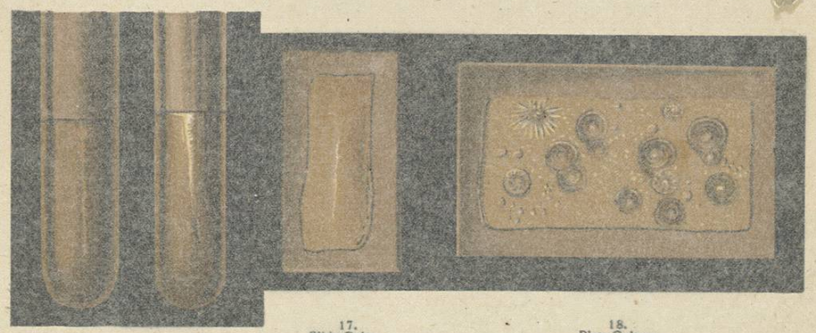
3. *Absorption of Oxygen.*—The most convenient absorbent for this purpose is an alkaline solution of pyrogallic acid. The principle was first utilized by Buchner for tube cultures, as shown in Fig. 5095. The large outer tube is provided with a closely fitting rubber stopper. On the bottom of the tube is placed about a gram of pyrogallic acid and a suitable support. The tube containing the nutrient medium is inoculated in the usual way and placed on this support. Finally 10 c.c. of a ten-per-cent. solution of potassium or sodium hydrate is added from a pipette, as rapidly as possible, and the tube is then quickly closed with the stopper.

As mentioned above, the Novy apparatus can be employed for the pyrogallate method. In the case of the jar a wide tube is introduced which contains about 2 gm. of the acid. After the culture tubes have all been inserted, about 20 c.c. of a twenty-five-per-cent. solution of sodium hydrate is introduced into the pyrogallate tube



1. Bacillus of Tuberculosis.
2. Bacillus of Cholera Asiatica.
3. The same in gelatine.
4. Finkler-Prior's Comma Bacillus.
5. Deneke's Bacillus
6. Miller's Bacillus.
7. Bacillus of Typhoid Fever.

8. Pneumococcus.
9. Bacillus of Glanders.
10. Bacillus of Anthrax.
11. The same in gelatine.
12. Bacillus of Malignant Oedema.
13. Bacillus of Septicaemia of Mice.
14. Bacillus of Septicaemia of Rabbits.



15. Bacillus of Chicken Cholera.
16. Bacillus of Pigeon Diphtheria.
17. Slide Culture (reduced).
18. Plate Culture (reduced.)

TEST-TUBE CULTURES.

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

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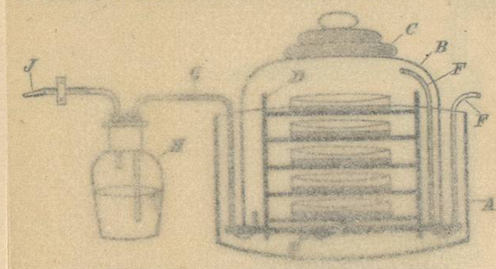

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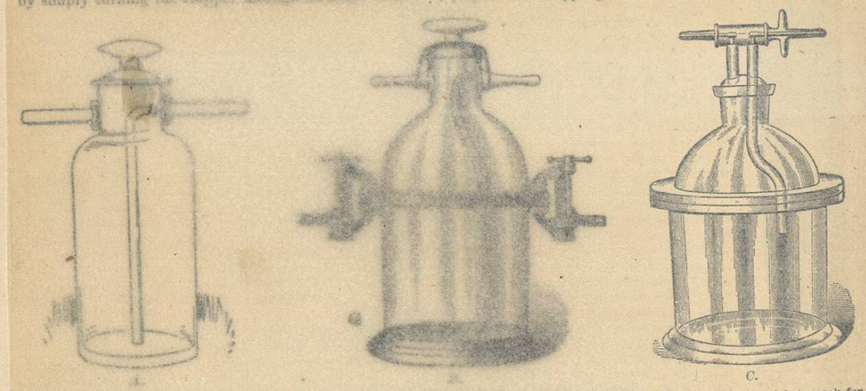

FIG. 5093.—Novy Apparatus for Anaërobes. *A*, Bottle for tube cultures; *B*, for for Petri plates; *C*, jar for plates with special stopcock for vacuum culture.

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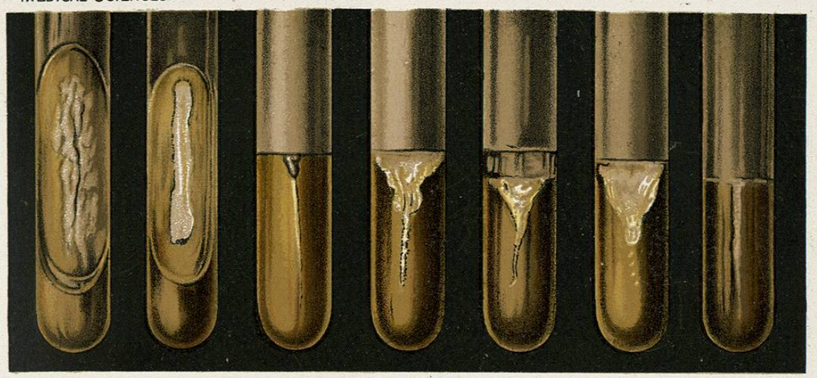

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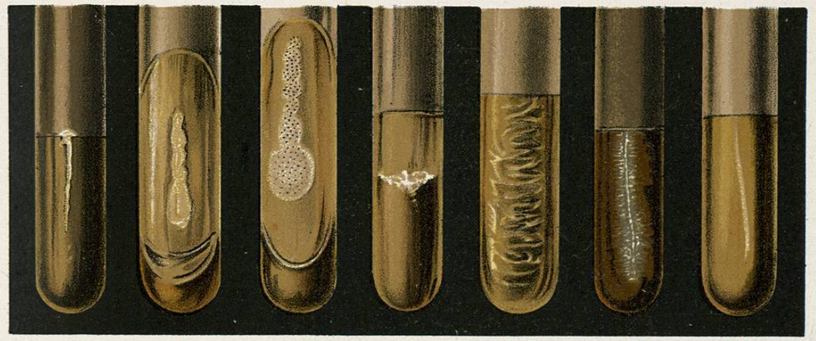

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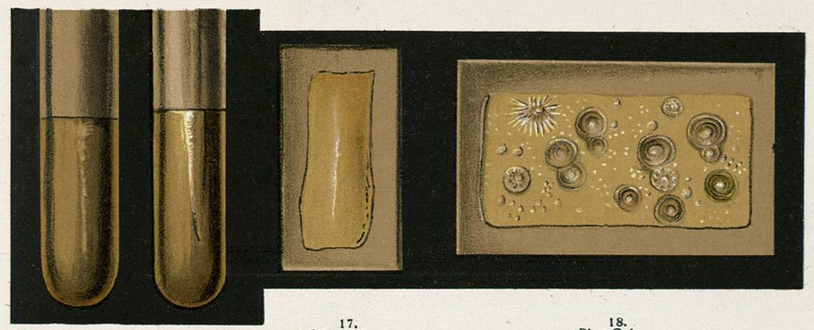

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TEST-TUBE CULTURES.

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from a pipette, and the stopcock is then inserted as quickly as possible and turned. In the case of the plate apparatus a crystallizing dish, about 10 cm. in diameter and about 2 cm. high, is placed on the bottom, and about 4 gm. of pyrogallic acid added. A couple of strips of glass are then placed on top, and on these are stacked the Petri dishes. Twenty-five cubic centimetres of the strong alkali are introduced in the manner just mentioned, after which the top is put into place, the clamps and the rubber band are applied.

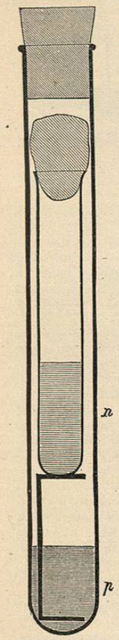

FIG. 5095.—Buchner's Pyrogallate Method.

Wright's method will be found to be very useful for occasional cultures. The cotton plug is cut off square and pushed down about 1 cm. into the tube. The plug is made of absorbent cotton. It is then moistened with about 4 c.c. of a strong solution of pyrogallic acid, after which about the same volume of strong sodium hydrate (1:2) is added. The tube is closed as rapidly as possible with a tight-fitting rubber stopper. Obviously, solid pyrogallic acid may be used.

It will be seen that the pyrogallic-acid method is extremely convenient and very simple, requiring a minimum of time and expense. The method is also applicable for the *hanging-drop examination* of anaërobic bacteria. A drop of alkali and of the acid can be placed on the side of the concave slide. After the cover is in place the slide can be tilted so as to bring the two liquids together. A special slide for this purpose was devised by Braatz (Fig. 5096).

The hanging drop is made and placed over the well. The flat flask contains the pyrogallic acid and communicates with the space below the drop. Strong alkali is finally added and the flask is closed with a stopper. Pyrogallic acid can also be employed in connection with Hill's "hanging-block" culture. Another apparatus for anaërobic hanging drops is that of Kühne. It is very serviceable and can be used for either the gas or pyrogallate process.

4. *Exhaustion of Air.*—Pasteur employed **U**-shaped tubes, from which the air was removed by means of an air pump. Gruber applied the principle to the tube culture. He used a stout glass tube, which was provided with a stopper, through which passed a short glass tube by which connection was made with the air pump. The test tube was constricted just below the stopper so as to facilitate the subsequent sealing process. The tube was filled in the usual way with the nutrient medium and inoculated. It was then connected with the pump, and as soon as the air was exhausted the tube was sealed at the constriction. The plate apparatus shown in Fig 5094, *C*, is intended for vacuum as well as gas or pyrogallate cultures. It can be used for tube or plate cultures.

5. *Mixed Cultures.*—This method of cultivating anaërobic bacteria corresponds to the way in which these organisms grow in nature. If the anaërobe is planted together with an aërobe, the latter will consume all the oxygen in the immediate neighborhood, and as a result the anaërobe will grow. Thus, if the tetanus and hay bacilli are planted at the same time into a tube of bouillon, they will both develop. Other aërobic bacteria, such as the Bacillus prodigiosus and Proteus vulgaris, can be used for the same purpose.

6. *Cultivation in Air.*—This of course is apparent rather than real. If a tube of glucose gelatin, preferably colored with litmus, be inoculated with an anaërobe and then set aside in the incubator, an abundant growth will develop (Novy's method). Similarly, when deep stab cultures are made of the anaërobes, it will be found quite frequently that the water of condensation on the top of the medium is cloudy from the growth of the germs. The explanation in the one case is that air is excluded partly by the viscosity of the liquid and partly by the evolved gases. The gas formation accounts for the growth of the germs in the water of condensation. The culture in glucose litmus gelatin is by far the simplest way of growing anaërobes. Moreover, the cultures thus obtained retain their vitality for many years. In some cases the author has recovered cultures from tubes five and six years old.

Collodium Sacs.—This method of cultivating has been used extensively by the Pasteur School for exalting the virulence of bacteria. The underlying idea is to grow the organisms in the peritoneal cavity of an animal, and under such conditions that the waste products of the germs will be removed, an abundant supply of nutrient material furnished, and the germs themselves protected against the action of phagocytes. This is accomplished by enclosing the bacteria in a hermetically sealed sac, the walls of which are permeable to the waste products of the germs and to the soluble proteids of the peritoneal fluid. Several Russian workers have employed for this purpose the inner lining membrane of eggs, but the best procedure is to make the sacs of collodium. Various methods have been devised for the rolling of the sac, but undoubtedly the best and simplest is that perfected in the author's laboratory by Gorsline.

The rolling tube employed for making sacs is about twelve to fifteen inches long, and of any width that may be desired. For ordinary purposes a width of half an inch is sufficient. One end of this tube is rounded off like a test tube, and has a 2 mm. opening at the tip. This opening is first closed with collodium either by touching it with the cork which has been covered with the solution, or the collodium may be applied with the finger. Care must be taken to see to it that the collodium does not get inside of the tube. In a few seconds the layer is dry enough to go ahead.

The collodium used is the United States Pharmacopœia solution, which by exposure to the air has been concentrated by one-third or one-half. It should be perfectly clear, and if not it must be filtered through cotton by the aid of a pump. The collodium can be kept in a glass-stoppered cylindrical vessel, such as is used for the collection of blood. The collodium is inclined till it comes within a few inches of the opening.

The rolling tube, with the opening freshly closed, is dipped in the collodium and rolled several times in the liquid. It may be rolled so that only the lower side of the tube touches the collodium. If the sac is to be very thin it is sufficient to roll the tube but two or three times, after which it is raised from the liquid and rolled in the ether atmosphere in a horizontal position till the collodium has set. If the layer is not thick enough the tube can be returned to the collodium, but care must be taken

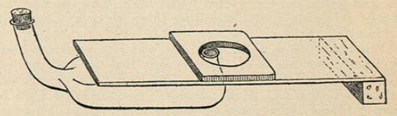
FIG. 5096.—Braatz's Slide for Anaërobic Hanging-drop Examinations.

to avoid the formation of air bubbles. The coated tube is finally rolled in the air until it has reached the proper consistence. This can be ascertained by touching the thickest part with the finger. The collodium layer should be rather firm. The tube is then immersed in distilled water for a minute or two. If the collodium is not sufficiently hard, it will cloud or become milky on contact with the water. It should remain perfectly clear, and when finished a thin sac placed in water is almost invisible.

To detach the sac the tube is filled with distilled water, and by blowing into the open end the water can be forced through the opening below and upward between the sac and the tube. By slight manipulation with the fingers the detachment can be effected readily on all sides. The free end is then trimmed square, after which the sac is placed in distilled water, where it remains until it is ready to be attached to the glass tube.

An ordinary test tube having a diameter slightly less than the sac is constricted in the blast flame at about two inches from the end. A scratch is then made, about a half an inch below the constriction, with a diamond, and with the aid of a hot rod the end is removed. The resulting tube has the form shown in Fig. 5097, *a*. The cut end should be rounded in the flame so as to remove the sharp edge.

The inside of the neck of the sac is dried by means of filter paper, after which the end of the tube is inserted. This can be done more easily if the end of the tube is previously dipped in alcohol. The next step, that of shrinking the sac upon the tube, is very important and requires care. Most of the shrinking is done by rotating the tube, in a horizontal position, some distance above a small spare-flame burner. In this way the collodium can be made to contract down upon the glass, but the operation must be done slowly and at some distance above the flame, otherwise there is danger of igniting the sac. The adhesion is rendered more complete by the application of a hot glass rod. Finally a silk thread is wound as closely as possible over the glass neck, and this in turn is covered with a layer of collodium. The sac now has the appearance shown in Fig. 5097, *b*. The finished sac is now filled with distilled water and placed in a test tube on foot, which also contains water (Fig. 5097, *c*), and the whole is sterilized by steaming in an autoclave for half an hour at 110°.

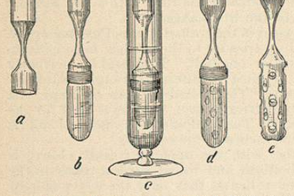

FIG. 5097. — Preparation of Collodium Sacs. (Novy.)

When the sac is to be used, the water is removed from the inside by means of a drawn-out pipette and refilled in like manner with bouillon which has been inoculated with the organism to be tested. The threaded part is then wrapped in a piece of sterile filter paper, for convenience in handling, and the constriction is sealed in a sharp-pointed flame. The sealed sac is then placed in a sterile test glass.

The rabbit or guinea-pig which is to receive the sac is now attached to a holder and the hair is removed from the abdomen. The field of the operation is thoroughly washed with lysol or mercuric chloride. After the animal is anæsthetized an incision is made in the abdominal wall, and through this the sac is introduced into the peritoneal cavity. The incision is then sewed up and covered with cotton and a little collodium.

The sac is allowed to remain in the animal for a few days or even for several months. To remove it the animal is killed with gas. The sac is freed from the adhesions and transferred to a sterile test glass with the glass end downward. By means of a hot rod an opening is burned into the end of the sac, and through this the contents are removed by means of a drawn-out tube pipette. When large sacs are to be inserted into an animal it is advisable to strengthen these by placing within a perforated glass tube as shown in Fig. 5097, *e*, *f*.

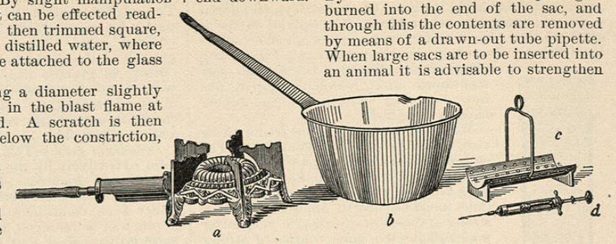

FIG. 5098.—Syringe Holder and Sterilizing Pan. (Novy.)

The collodium sacs can be used not only as just described, but also with marked advantage for dialyzing experiments. For this purpose the sacs can be made an inch or more in diameter and twelve or fifteen inches long. The thin collodium membrane is considerably more permeable than parchment paper.

INOCULATION OF ANIMALS.—According to the nature of the experiment these are made with pure or impure cultures of bacteria, or with the chemical products elaborated by them. The use of impure material is met with in diagnostic work. Thus in suspected glanders the discharge is introduced into animals in order to ascertain if the bacillus of glanders is present. The same is often done in tuberculosis, pneumonia, bubonic plague, anthrax, tetanus, rabies, etc. In all these experiments the animal serves as a plate, since it eliminates all the saprophytic bacteria which may be present in the original material and allows the disease-producing ones to develop in pure or almost pure cultures. The inoculation with pure cultures is made to test their identity, to study their effects upon animals, and to ascertain the diverse means of infection, and for purposes of immunization. The inoculation with the chemical products serves to ascertain the presence of poisonous substances, or to produce vaccines or antitoxins.

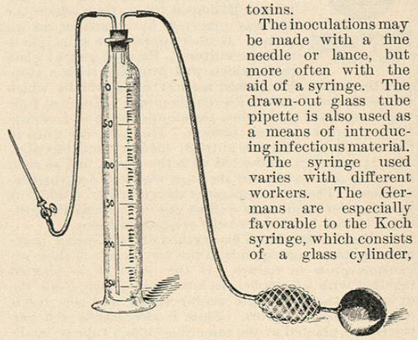

FIG. 5099.—Graduated Cylinder for Injecting Liquids. (Novy.)

The inoculations may be made with a fine needle or lance, but more often with the aid of a syringe. The drawn-out glass tube pipette is also used as a means of introducing infectious material.

The syringe used varies with different workers. The Germans are especially favorable to the Koch syringe, which consists of a glass cylinder, graduated, the narrow end of which connects with the needle while the upper end fits into the metal collar of a rubber bulb. The advantage claimed is that the cylinder and needle can be effectively sterilized by dry heat. As a matter of fact the Koch syringe is extremely inconvenient and unsatisfactory, and equally good results with less time and annoyance are obtainable with the ordinary hypodermic. The latter of course must be sterilized by boiling in water for ten or fifteen minutes. A convenient holder for the syringe is shown in Fig. 5098.

When large quantities of liquids are to be introduced, as when injecting horses with diphtheria toxin in the preparation of antitoxin, an apparatus similar to that shown in Fig. 5099 can be used.

The necessary instruments, such as knives, scissors, needles, etc., are sterilized by boiling in water, or better in a saturated solution of borax. A very convenient sterilizer for this purpose is that shown in Fig. 5104.

In all operations the animal must be secured in some way or another. Various kinds of holders have been constructed for this purpose. That of Latapie, shown in Fig. 5100, is very convenient, and is to be preferred to the ordinary models. It can be used for guinea-pigs, rabbits, birds, etc.

The Voges holder, shown in Fig. 5101, is useful for taking temperatures and for injecting small animals. A good substitute can be made by using a glass cylinder.

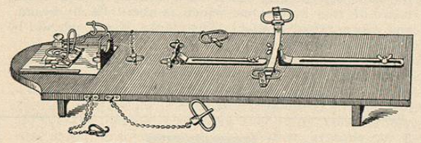

FIG. 5100.—Latapie's Animal Holder. (Novy.)

Special holders have been devised for rats and mice. These, however, can be handled best by means of a pair of compression or artery forceps. The animal is seized by the nape of the neck with the forceps, which are then transferred to the left hand. The tail and the hind legs are also held by this hand. The animal in this way is put upon the stretch, and the inoculation can then be made with the right hand. Even full-grown wild rats can be handled in this way without the help of an assistant.

FIG. 5101.—Voges' Holder for Small Animals. (Novy.)

After inoculation the animals are placed in special jars or cages. The ordinary glass battery jars, provided with a galvanized wire top, weighted with lead, serves to confine rats and mice, and can even be used for guinea-pigs (Fig. 5102). If the animals are inoculated with a very dangerous organism, such as the pest bacillus, it is advisable to place the jar inside of a ten-gallon crock. In special cases, as in animals infected with trypanosomes, bubonic plague, etc., it is well to cover the cage with a piece of mosquito netting as a safeguard against insects spreading the infection.

Guinea-pigs, rabbits, and the like can be kept in the Vaughan cage shown in Fig. 5103. The cage proper is 30 cm. high, 38 cm. deep, and 54 cm. wide. The feet are 12 cm. high.

1. *Cutaneous Application.*—Ordinarily bacteria do not penetrate the unbroken skin or mucous membrane. The direct application of some organisms, even in the absence of any known lesion, leads to infection. This is the case when the virus of the foot-and-mouth disease or the bacillus of plague is brought into contact with the mucous membrane. The pus germs, when rubbed into the skin by the aid of vaseline, may cause infection.

2. *Subcutaneous Application.*—For this purpose the hair is removed from the region where the inoculation is to be made. The place is then rubbed with a disinfectant. In the rat this is usually on the back, at the root of the tail; in the guinea-pig it is on the side. A nick is made with sterile scissors, and then with a narrow scalpel or spatula a pocket is made under the skin. A piece of tissue, a bit of earth, blood-laden wire, etc., is then introduced into the opening, which if made small requires no special closure.

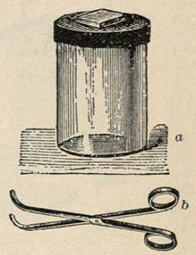

FIG. 5102.—Rat Cage and Forceps. (Novy.)

3. *Subcutaneous Injection.*—The suspended material is introduced under the skin by means of a syringe. The hair should first be clipped close and the place of inoculation touched up with a disinfectant.

4. *Intravenous Injection.*—In the case of the rabbit this is easily done. The marginal branch of the posterior auricular vein is selected, although it may appear to be narrower than the needle. The hair may be removed and the surface of the ear rubbed freely to stimulate circulation. A clamp is then applied at the base of the ear so as to distend the vein. The needle is then inserted at a very slight angle to the vein. In other animals the jugular can be exposed and the injection made without any difficulty. In the preparation of preventive sera the culture or toxin is often injected into the jugular of a horse. A trocar is first introduced into the jugular, and this is then connected with the cylinder containing the liquid to be injected (Fig. 5099).

5. *Intraperitoneal Injection.*—This procedure is very commonly resorted to. The skin over the abdomen should be raised and the needle of the syringe is then introduced into the cavity. In the case of the horse, while the animal is standing, a trocar is introduced through the skin at a point a few inches anterior to the crest of the ilium.

6. *Intrapleural Injection.*—The needle is introduced into the right pleural cavity, care being taken to prevent any injury to the lung or to the heart. Large amounts of liquid cannot be tolerated by the animal.

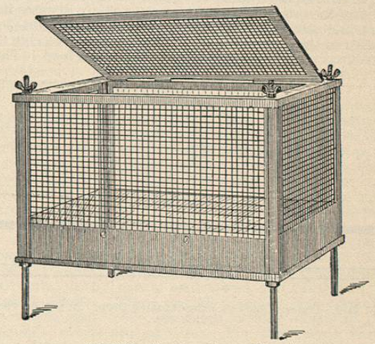

FIG. 5103.—Vaughan Cage. (Novy.)

7. *Intracranial Injection.*—This method was introduced by Pasteur as a means of surely infecting animals with rabies. The procedure is followed out when inoculating animals either for diagnosis or for the purpose of preparing the vaccine for hydrophobia. It is usually practised on rabbits and guinea-pigs. The skin from between the ears forward is shaven clean and disinfected. An incision about an inch long is then made. The Pasteur School apply a hand trephine, and make an opening into the skull. A small trephine, operated by a dental engine, is much more convenient. In the absence of either an opening may be made into the skull with a stout scalpel. By means of a hypodermic syringe a few drops of the brain or cord suspension are then introduced under the dura. At times the injection is made into the brain proper, in which case it is spoken of as *intracerebral*. After the injection a suture or two is applied, and the wound is covered with collodium and cotton.

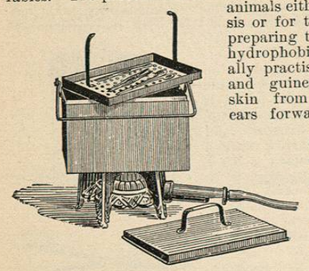

FIG. 5104.—Instrument Sterilizer. (Novy.)

8. *Intra-ocular Injection.*—Cocaine is first applied to the eye, after which this is fixed with forceps and the material injected into the anterior chamber. If desired, an opening can be made with a cataract knife or narrow scalpel and the solid material can be introduced in this way.

9. *Injection into the Lymphatics.*—This is usually made by introducing the material into the testicles.

10. *Respiratory Infection.*—While the preceding methods may be looked upon as wound inoculations, this concerns itself as nearly as possible with duplicating the natural infections along the respiratory tract. The direct method consists in causing the animal to inhale the finely divided material, which can be readily done by means of an atomizer. Another procedure may be called the *intratracheal* injection. This is carried out by making an opening into the trachea, and through this introducing the infectious agent.

11. *Alimentary Infection.*—Since water and food serve to introduce the pathogenic agent of many diseases into man and animals, it is necessary at times to resort to a similar method of infection. The animal may receive the infectious agent in water, milk, or in solid food. Thus

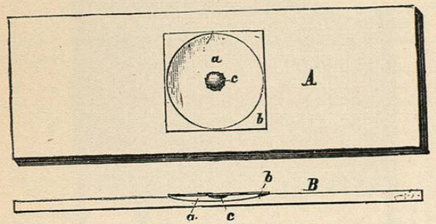

FIG. 5105.—Concave Slide showing Hanging Drop. *A*, Surface view; *B*, side view.

bread may be soaked in a bouillon culture of the organism. At other times it may be necessary to introduce the material into the stomach by means of a rubber tube. In order to prevent the animal from biting the tube, it is well to pass it through a perforated cork or plug of soft wood. Under exceptional conditions a laparotomy may be made and the material injected into the intestines. This is spoken of as the *intraduodenal* injection.

Observation and Autopsy of Infected Animals.—The matter of suitable caging of animals has already been touched upon. Attention may be called to the need of daily observations of the infected animals, so as to note the symptoms manifested. The animals must have plenty of food and drink, and must be kept in as clean a condition as possible. Their weight and temperature should be taken daily, for in this way the best information can be gained as to the physical condition of the animals.

When the animal dies it should be autopsied at once, or else it must be put aside in an icebox. The need of immediate examination is shown in some of the trypanosomatic infections, as nagana and caderas, where the organism may disappear from the blood within an hour or so after death. Moreover, delayed examination may lead to the invasion of the organs of the cadaver by the intestinal bacteria, in which case the search for the specific germ is rendered more difficult, if not impossible.

The animal is prepared for autopsy by being placed on its back and tacked down on a board. A convenient board of this kind is one which is about 34 by 54 cm., and has a raised border. The cracks, if any, should be filled with paraffin.

After the animal is laid out, the hair should be thoroughly moistened with mercuric chloride solution. The

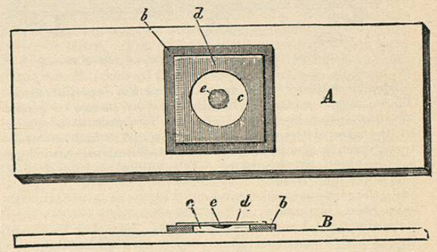

FIG. 5106.—Cell Slide showing Hanging Drop. *A*, Surface view; *B*, side view; *b*, edge of cell; *c*, hollow of cell; *d*, cover glass; *e*, hanging drop.

necessary instruments can be sterilized in a copper sterilizer, such as is shown in Fig. 5104. In the absence of such an arrangement the instruments may be sterilized by heating directly in the flame, but this, of course, injures them. A searing iron, several drawn-out pipettes and sterile dishes, as well as the necessary media, should be conveniently on hand

With a sterile scalpel an incision is made along the entire length of the body from the neck to the pubis. Lateral incisions are then made in the direction of each of the extremities, and the two large flaps thus resulting are turned back. The condition of the subcutaneous tissue, the presence of œdema, bloody effusions, enlarged lymphatic glands, etc., are noted. The glands or portions of the tissues may be transferred by means of sterile instruments to a sterile dish. Cover-glass smears or streaks can be made and examined either at once or later.

The abdominal and thoracic cavities are usually opened at the same time. The abdominal wall in the lower part of the body is slightly raised and nicked with sterile scissors; then the lower blade is inserted and the incision prolonged upward to the diaphragm. The ribs are then cut as low down as possible, and the wedge-shaped piece of the wall of the thorax is removed. The condition of both cavities and of the organs is carefully noted. Cover-glass streaks are made from the peritoneal surfaces and from the cut surfaces of the organs, and examined either at once or later. Any fluid which is present in

the cavities may be transferred to sterile tubes by means of the pipette.

Cultures should always be made from an intact organ. For this purpose it is cut open with sterile scissors, and a piece of the pulp removed on a sterile wire, or by the aid of a Nuttall spear or spatula (Fig. 5083). The heart blood is usually given preference for culture purposes. The pericardium should be opened, after which the surface of the heart is seared with a hot iron. An incision is then made into the ventricle, from which the blood can be removed by the aid of a looped wire. The best way of removing the heart blood is by means of a sterile Pasteur bulb pipette. The end of this is broken, flamed, and when cool it is inserted into the heart, and by suction the blood is drawn up into the pipette. The contents of the tube can then be used to inoculate culture media or for making blood streaks.

After the autopsy the animal should be placed in a vessel and steamed or autoclaved, and eventually burned. The board should be washed with mercuric chloride, and all instruments and utensils should be sterilized by steaming. Throughout the autopsy care must be taken to prevent infection, either by scattering the material on the floor or by its being carried away by insects.

EXAMINATION OF BACTERIA.—In order to gain some definite information regarding the bacteria which develop on the nutrient media, or in the animal body, recourse must be had to the microscope. The organisms may be examined in the living condition or in stained preparations. The former procedure is resorted to so as to learn all that is possible regarding the living cell: its form, size, color, granulations if any, motion, grouping of the cells, presence of spores, etc. Such facts are ascertained by making a preparation in which the bacteria will continue to live for some time.

Living Bacteria.—1. The simplest procedure is to place a drop of the bacterial liquid on a slide, after which the cover-glass can be applied and the preparation examined under the microscope. This method is useful for rapid orientation, but it has certain drawbacks, chief among which is the fact that evaporation takes place along the edge of the glass, and as a result currents are established in the liquid. Such currents tend to interfere with the observation of any one organism or group of cells. Again, a preparation of this kind cannot be kept under observation for any length of time on account of the desiccation which soon takes place.

2. The examination in a hanging drop, as it is called, obviates the difficulties mentioned. A rather thick slide with a concave well is used (Fig. 5105). A ring of vaseline is spread around the edge of this well. A clean cover glass, about three-fourths of an inch in diameter, is placed on the table, and a drop of water is applied to the middle by means of a looped platinum wire. It is desirable that the drop should spread out flat, and if it does not it is because the cover glass is not clean. The drop of water is then inoculated with a little of the culture. Just enough material is added so that the liquid is slightly cloudy. The vaseline-ringed slide is then inverted and brought down upon the cover glass. The preparation is turned over, and, if need be, pressure applied to the border of the glass so as to have an air-tight hanging drop. Under these conditions evaporation does not take place, and consequently the specimen may be examined for hours, if necessary, without any interference by currents due to evaporation. As mentioned above, this method can be used for the cultivation of bacteria, and thus their growth and multiplication can be followed out. In that case it is necessary to use a flamed cover glass and a sterile liquid.

Instead of the concave slide a so-called well slide can be used (Fig. 5106). This is essentially a square bit of glass with a circular opening which is cemented to an ordinary glass slide, and the hanging drop is then made in the manner described.

One disadvantage in either method lies in the fact that the drop is more or less convex, and consequently when using higher powers it is difficult to examine the deeper portions. This difficulty can be overcome by employing the Ranvier slide, which has a circular trough, and the portion within the circle is ground down so that its level is about 0.1 mm. below that of the slide. When a drop of liquid is placed within the circle and covered with a cover glass, the liquid spreads out into a thin layer, every part of which can be examined under the microscope. A ring of vaseline is placed along the edge so as to prevent evaporation. By flaming the slide and cover slip and using sterile liquid the preparation can be observed for several days if need be. This method is especially to be recommended for studying trypanosomes, malaria parasites, etc.

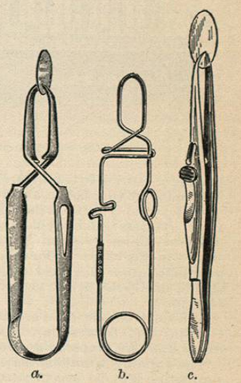

FIG. 5107.—Cover-glass Forceps. *a*, Cornet's; *b*, Stewart's; *c*, Novy's.

Staining of Bacteria.—In order to obtain good stains it is necessary to have good clean cover glasses. The cover slips, as purchased in the market, are unfit for use until they have been cleaned. One method of doing this is to heat the slips in a beaker with concentrated sulphuric acid and potassium bichromate. The cover glasses are then washed in running water, after which they are kept in alcohol. Another procedure which gives very satisfactory results is to soak the cover glasses first in alcohol, after which they are wiped with soft, washed linen, placed in an Esmarch dish and heated in a dry-heat sterilizer at about 200° for an hour or two. This high heat completely destroys the organic matter that may be on the glasses. A cover glass is not clean if a small drop of water, when spread over the surface, does not remain even but gathers into droplets.

Several kinds of forceps have been devised for holding cover glasses while staining. The Cornet forceps (Fig. 5107, *a*) are well known, and are useful though rather awkward. Stewart's modification is widely used (Fig. 5107, *b*). A much more convenient forceps is shown in Fig. 5107, *c*. The lower blade has a thin edge which permits one to pick up the cover glass without contact with the fingers. The upper blade is bent in order to avoid capillarity, and is narrowed to a point so that the specimen is held by point contact. A catch serves to hold the cover slip in place.

Aniline Dyes.—The aniline dyes which are employed for staining purposes are either basic or acid in character. The former contain amido groups and are spoken of as nuclear stains, since they color the nuclei of cells as well as bacteria. The latter contain hydroxyl groups and do not stain bacteria, but are used chiefly for contrast coloring, and to some extent for decolorizing. The basic dyes are usually employed as salts of hydrochloric acid, while the acid dyes occur as sodium or potassium salts.

Among the basic aniline dyes which are commonly employed may be mentioned fuchsin, gentian violet, methyl violet, crystal violet, methylene blue, thionin, safranin, methyl green, neutral red, and vesuvin or Bismarck brown. These are all more or less crystalline powders, and while some are definite chemical compounds, others are mixtures. For this reason various brands are met with on the market, and it will be readily understood why the exact duplication of stains is not always possible.

It is advisable to keep on hand not only the solid dyes, but also stock solutions which are saturated alcoholic