SOME SIMPLE ANAEROBIC METHODS.*

BY

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(From the Pathological Department, Guy's Hospital.)*(Preliminary Communication.)*

THE methods usually employed for the cultivation of anaerobic organisms are not ideal. Considerable time and trouble must be taken and elaborate apparatus employed if a fair degree of anaerobiosis is required. The methods are not cheap and the apparatus is bulky. It was hoped that the existing methods might be greatly simplified and the attainment of anaerobic conditions greatly hastened by the employment of quite simple apparatus. It is felt that this end has been attained, and although the experiments are not yet complete and it is not beyond all doubt which of the methods to be described below will ultimately prove the best, it is thought advisable to publish a brief account of the methods in order that other people may have an opportunity of trying them. This seems the more advisable as the methods are so simple that they might be used in a travelling laboratory, and so might be of service to those who are working with anaerobic bacteria in the field, for those whose space is limited, and who cannot take a large equipment with them.

The methods are all very simple, and most of them require only the usual media which are used in any bacteriological laboratory. They have been tested on such anaerobic bacteria as *B. botulinus*, *B. tetani*, and the bacillus of malignant oedema. They all depend on the properties which finely divided metals possess of adsorbing gases and other simple substances.

METHOD 1.—*Porous Platinum.*

This method is the simplest and is applicable to solid or liquid media, and for these reasons it will probably prove the best.

Short pieces of platinum wire are fixed into glass holders at the blow-pipe and the free ends are wrapped tightly round small pieces of gas carbon, or other porous material which will char readily, and secured by twisting round the main piece of wire. These pieces of carbon are then heated in the flame from a Bunsen burner to expel the air and dipped while still hot into a strong solution of platinic chloride. After soaking for some time they are removed and dried over the flame. They are then heated red hot and redipped and the process repeated two or three times. It will be found that, on removal from the flame, the reduced platinum on the surface of the carbon will adsorb sufficient oxygen from the air to keep the mass a dull red until all the carbon is burned away.

We have now several pieces of porous platinum mounted on wire and fixed to glass holders. For use the glass is cut short and pushed into the centre of a cork. With these and a Kipp's hydrogen apparatus it is possible to render the atmosphere above a medium anaerobic in a few minutes.

It is particularly suited to the cultivation of anaerobic organisms on small amounts of solid media. Suppose that an anaerobic culture is desired on a blood agar slope in a test-tube. The tube is infected in the usual way. It is turned upside-down and the cotton-wool plug removed. A sterile glass capillary tube connected with the hydrogen apparatus, and with a cotton-wool plug in it, is introduced from below, and a brisk stream of hydrogen run into the test-tube. No precautions are taken to prevent the hydrogen diffusing out of the tube except that the remaining operations are carried through rather quickly. The capillary is removed and the platinum armed cork, which has been sterilized by passing through a flame, is pushed home. If an ordinary cork is used it is advisable to paint the joint with melted paraffin wax. If the right amount of hydrogen has been introduced the platinum will glow dull red as soon as it is introduced and continue to do so until all the residual oxygen is used up in forming water. If too little hydrogen has been led into the tube the platinum will become white hot, and an explosion will

result. It is advisable, then, that sufficient hydrogen be passed into the tube in order to prevent this happening. If the procedure has been carried through properly the atmosphere in the tube is now devoid of oxygen and the whole may be incubated. As oxygen in the blood agar is given off one can see the haemoglobin become reduced by the colour change and confirm this with a spectroscope. The deeper layers of the medium give up the oxygen slowly, and some hours elapse before the whole is reduced. This, however, does not matter, since the surface on which the growth is required is anaerobic in a few minutes. As oxygen is given off from the medium it is adsorbed by the platinum and burned to water, as in the first instance.

Using this method the organisms of tetanus, botulism, and malignant oedema grew with great freedom, and nearly the whole surface of the agar was covered with growth in forty-eight hours. Transplants from these gave visible growth in fourteen hours.

A similar procedure can be used for plating methods if flat bottles, similar to the Roux pattern, are used. The procedure is the same as for test tubes, except that it is advisable to use larger pieces of platinum and to use plenty of hydrogen. Explosions in a bottle might be dangerous. It may be mentioned in passing that it is necessary to allow several minutes to elapse after one of these bottles filled with hydrogen has been opened before introducing a warm platinum loop to take out a sample. A warm platinum loop on one occasion caused an explosion which, though it did no harm, was rather startling. It is better to use large pieces of platinum, because they warm up more slowly and they have a bigger surface. It is essential that the platinum be introduced while still warm, as if cold, the water formed may not evaporate, and if the pores of the spongy platinum become waterlogged the adsorption of gases and their union is very slow. It is quite simple to guard against these sources of error by using plenty of hydrogen and seeing that the platinum glows dull red when it is introduced.

With this method all anaerobes tested grew quite well, and it was found possible to isolate an anaerobic organism from faeces which has not yet been fully identified.

The method can be applied to broth. In this case, since the tube cannot be held upside-down, a very brisk stream of hydrogen is bubbled through the medium and the tube corked up as soon as possible after the capillary is withdrawn.

In every case a little oxygen should be left inside the vessel and the platinum should glow when introduced. In this way a small negative pressure is made inside the vessel, and room given for the expansion of gases on incubation, and for any gas which may be formed by the growing micro-organisms. There is thus no tendency for the vessel to burst or the cork to be blown out.

It will be seen that the method is very simple in theory and in practice. It is also cheap, since the estimated cost of the platinum is threepence to a shilling, and the pieces can be used again and again. Some experiments were carried out with palladium, but no advantage was found. The method is very rapid. A tube or bottle can be infected, rendered anaerobic, and put in the incubator in a few minutes.

METHOD 2.—*Colloidal Platinum.*

Test-tubes are taken, and into each there is introduced a second test tube which is cut off short. The inner tube nearly fills the lumen of the outer, and it is introduced upside-down. These tubes are filled out with broth, to which a trace of methylene blue has been added. The tubes are then plugged with cotton-wool, sterilized, and kept ready for use. When an anaerobic culture is desired 2 c.cm. of a platinum sol are added, the tube infected, and a stream of hydrogen passed through the upper layers of the medium by means of a fine capillary. It will be observed that the methylene blue is gradually bleached. The colloidal platinum acts as a catalyst, and the hydrogen destroys all the oxygen present in the mixture. When this stage is reached the capillary is passed down to the bottom of the tube, and the inner tube filled with hydrogen. This then rises to the top of the fluid and acts as a gasometer. The capillary is withdrawn, and a cotton-wool plug inserted, and the whole incubated. The gasometer of hydrogen supplies a reducing agent which will destroy the oxygen absorbed from the surface of the medium provided

* The expenses of this research have in part been met from a grant from the British Medical Association.

that sufficient platinum is present. The method gave good results with the organisms of botulismus and malignant oedema, but with tetanus the growth was slow and poor.

METHOD 3.—Colloidal Platinum and Sodium Formate.

An attempt to dispense with hydrogen was made by adding to broth excess of sodium formate and colloidal platinum. In a system of this kind it was expected that all the free oxygen would be used up in oxidizing the formate owing to the vigorous catalytic action of colloidal platinum. This expectation was not fulfilled completely. The addition of colloidal platinum to a solution of sodium formate in water containing a little methylene blue brings about complete destruction of all the free oxygen in a short time, and this whether the solution be acid, neutral, or alkaline. The anaërobic state of the solution will continue for some time, for as soon as there is any oxygen absorbed it is used up in oxidizing the formate. But when the method is tried on broths the results are not nearly so good. Some constituent, in some specimens of broth, interferes with the reaction. Bouillon made with Witte peptone, for example, does not give good results. Broth made with a tryptic digest instead of Witte peptone (Douglas) was more suitable. To this medium there was added a trace of methylene blue, sodium formate up to 1 or 2 per cent., and just before infecting 2 c.cm. of colloidal platinum, or platinum black suspension. The infected tubes were plugged with cotton-wool, and thus there was free access of air to the medium. The tubes remained anaërobic for twenty-four to forty-eight hours, and, in cases where growth occurred, for all the time that growth was taking place. *B. botulinus* grew extremely well and regularly, the bacillus of malignant oedema less regularly, but when it did grow it gave a luxuriant growth. *B. tetani* did not grow well, and occasionally did not grow at all. It formed little toxin, and the bacilli stained badly.

After these methods had been devised, my attention was drawn to a paper by Pfühl,¹ who describes some methods of growing bacteria with free access of air. In one of these methods he adds 1 gram spongy platinum to bouillon, and boils out all air just before infecting. Care is taken not to shake the tube, and so allow the medium to take up oxygen. In this way anaërobic bacteria would grow. The exact part played by the platinum in his experiments is not quite obvious, as he does not seem to have added a reducing agent in order to make the reaction continuous. Moreover, the amount of platinum used is prohibitive, although it can be recovered.

In Methods 2 and 3 the platinum is never recovered, but as the weight of the metal thrown away is extremely small, the cost is not great. The platinum sol is made by making an arc between platinum electrodes under water. The sol is protected by the addition of a small amount of gelatine, and sterilized by repeated boiling and incubating.

The last two methods require further work to endeavour to get over the uncertainty and the poor growth of *B. tetani*. Moreover, the fact that some specimens of broth give poor results is a serious objection; and as the colloidal platinum is easily aggregated, and thus its efficiency as a catalyst destroyed, it may be that the difficulties will never be overcome. The colloidal metal seems to have no deleterious effects on the growth of the organisms, unless it is on this account that *B. tetani* does not grow very well. In the case of organisms of botulismus and malignant oedema, on the other hand, the growths are very good, and a rich crop results.

It seems from all the experiments so far carried out that the first method will prove the best, and the facts that it is so simple, can be used with all kinds of media, and that no addition or alteration of the medium is made in any way, seem to show that it will give the best results in the end.

REFERENCE.

¹ Pfühl, Die Züchtung von anaëroben Bacterien in Leberbouillon, sowie in Zuckerbouillon und in gewöhnlicher Bouillon mit einem Zusatz von Platinschwamm oder Hepin unter Luftzutritt. *Cent. f. Bact.*, Orig., xlv, 8, 378. 1907.

THE Pennsylvania Society for the Protection of Scientific Research has opened a campaign for the education of the public concerning medical research. The organization will include a branch consisting of medical practitioners who contribute £2 or more to further the objects of the society. Dr. William Pepper of Philadelphia is the Chairman of the Finance and Membership Committee.

THE THEORY AND TECHNIQUE OF TREATMENT BY RADIUM EMANATION NEEDLES.

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BRIEFLY, it may be stated that, for therapeutic purposes, radium *per se* is of no value; it is not till it breaks down and the third and fourth generations of the series of elements that come into existence are reached that therapeutic effects are produced. The therapeutic agents are the penetrating beta and gamma rays which are given off during the disintegration of radium B and radium C. The disintegrating radium atom at ordinary temperatures turns into a gaseous element—radium emanation—a gas that can be collected by means of a mercury vacuum pump and utilized as a much more convenient source of radium B and radium C than the solid radium salt.

The disadvantage of using a radium salt is that, owing to its great costliness, it cannot be changed from one form of receptacle to another to suit the requirements of different cases without serious loss of time and valuable material. This latter loss is more obvious when we consider that 50 mg. of radium chloride, which is at present worth at least £1,000, has approximately the same bulk as a grain of calomel.

The only serious disadvantage of radium emanation as a source of radiation is that, owing to there being no parent radium present to go on producing it, it is dissipated comparatively rapidly, its activity being reduced to half in 3.85 days.

At the end of any and every hour there is 0.75 per cent. less emanation present than at the beginning of that hour. This works out as a loss of 4.4 per cent. per six hours, or 16.5 per cent. per day, and of 71.6 per cent. per week. It must not be lost sight of that for the first four hours after emanation—without radium—is confined in a receptacle its activity increases—that is, till radium C equilibrium is established.

The advantages of radium emanation are: (1) It has a much smaller bulk than the parent radium salt. (2) It can be supplied from institutions at a reasonable cost, while for general use the price of radium is prohibitive. (3) It can be collected in capillary tubes, or flat applicators, or in any shape or size of glass or other vessel suitable for the requirements of the case to be treated, and this without risk of loss or permanent diminution of the value of the parent radium. (4) The loss of a tube of emanation is of comparatively little consequence.

Rationale of Radium Therapy.

In the treatment of deep structures, and especially malignant growths, the great advantage radium possesses over x rays is that the source of radiation can be inserted among the actual cells to be treated, and the rays, therefore, are not expended on the skin and healthy tissues before they reach their goal. By burying radium the cells that require radiation are primarily and principally affected, and protect the skin and healthy tissues by screening or filtering off from them the less penetrating rays. With the surface application of radium, or by using x rays, the reverse is the case: the skin receives a greater dose than the deep parts because it is nearer the source of radiation. For example, by using a soft and unscreened x-ray tube the skin may be burnt, while the deep structures, being so effectually screened by the superimposed tissues, may not be radiated at all.

In x-ray therapy this undesirable state of affairs is to some extent overcome by using a hard tube which will give off for the most part hard or penetrating rays, and also by interposing filters of various density and thickness, according to the depth of the tissues to be radiated. The filter cuts off the soft rays which would otherwise act on the superficial structures alone. The same consideration obtains in the surface application of radium, with this difference, that the quality or penetrating power of the rays from a given amount of radium is constant. The beta rays (corresponding to cathode rays) and gamma rays (corresponding to x rays) of radium B and radium C