

AN APPARATUS FOR ANAEROBIC PLATE CULTIVATION IN HYDROGEN FOR SEPARATE PETRI CAPSULES

(A REPORT TO THE FOOD INVESTIGATION BOARD)

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(With Plate IV.)

A NUMBER of methods have been described for anaerobic cultivation in separate plates. The principle offers certain advantages over the cultivation of a number of plates in the same anaerobic container, which has to be opened whenever it is desired to inspect any particular culture; and it is useful where anaerobic plate cultures are needed only occasionally or in small numbers.

A few methods are well known and have been widely used. These, however, depend upon alkaline pyrogallate for absorption of oxygen; and this principle is not always dependable, being especially liable to fail with light inoculations of pure culture of anaerobes.

A method was accordingly devised for single anaerobic plate cultivation in an atmosphere of hydrogen; in this the residual oxygen is removed by means of a heated capsule of asbestos-wool impregnated with carbonised palladium, after the method of McIntosh and Fildes.

A very perfect degree of anaerobiosis is thus quickly and conveniently obtained.

CONSTRUCTION OF APPARATUS.

The Petri capsule-holders used are made from the flanged metal tops of canisters in which jam and other commodities are commonly sold; following Henry's well-known device for single alkaline pyro-gallol plates. These canister tops consist of a flanged metal rim, with a central plate which must be removed to get at the contents of the canister. For the present purpose the flanged rim is cut off, and the central plate is soldered in position so as to be air-tight. The result is a shallow circular metal dish bordered with a wide groove which forms the Petri capsule plate-holder. To the back of this are soldered two short lengths of "white metal" piping of about 12 mm. external diameter. These are blocked at their inner ends, and their open ends project about half an inch beyond the rim of the plate-holder. Two small holes bored in the central portion of the plate-holder correspond to holes bored in the metal pipes close to their blind ends. The soldering of the pipes must be thoroughly done, so that the communications between the pipes and the interior of the plate-holder are perfectly air-tight.

Figs. 1 and 2 are photographs of the back and front, or external and internal aspects, of a plate-holder, and show its construction clearly. The

inner surface of the holder is japanned black to afford a dark background for colonies. Two sizes of plate-holder were used; one to fit the smaller half of a Petri capsule, and one to fit the larger half or cover. One Petri capsule was thus made to serve for two cultures. It was found easier to choose Petri capsules to fit plate-holders, than to choose tins whose flanged tops would fit Petri capsules. This is because Petri capsules of the same catalogued size are, nevertheless, variable in diameter; whereas tin canisters are made very accurately to certain measurements. Petri capsules were used in which the external diameter of the smaller halves was about 8 centimetres; these were catalogued as $3\frac{1}{2}$ -inch size, and both halves could usually be fitted to plate-holders made from easily obtained sizes of canister. To the open ends of the metal pipes pieces of glass tubing drawn out pipette-wise are attached by means of thick-walled (semi-pressure) rubber tubing. One of these pipettes—that for the hydrogen outlet—contains a cartridge about $1\frac{1}{2}$ inches long of “palladium-asbestos-wool” rolled in a single layer of copper gauze. The palladium-asbestos-wool (McIntosh and Fildes, 1917) can be obtained from Messrs Baird and Tatlock. It is best to lubricate the rubber tubing connections with a little vaseline.

Figs. 3 and 4 show the apparatus complete with colonies of *B. tetani* and *sporogenes*.

METHOD OF USE.

The separate halves of the Petri capsule are covered with the plain lids of cylindrical tin canisters which can be easily obtained of suitable sizes. They are sterilised in these covers and charged with nutrient agar in the usual way. Before inoculation, to secure discrete colonies, the agar plate should be dried for quite 2 hours in the incubator in the usual way by tilting it bottom upwards against the rim of its cover.

Two pieces of fresh plasticine are rolled into long “worms”; one of these is pressed well into the groove of the plate-holder so as to fill it completely. After inoculation and removing its tin cover, the plate is pressed firmly into its bed of plasticine, care being taken to apply pressure at the sides, and not near the centre of the plate, to avoid risk of cracking the glass. The second “worm” of plasticine is then applied to the junction of plate and holder, and thoroughly welted in and smoothed round to effect a perfectly air-tight connection. Dryness of the glass is essential for this, and may be secured if necessary by wiping with a cloth moistened with spirit.

The apparatus is rendered anaerobic by means of hydrogen delivered from a Kipps' apparatus. The inlet pipette—that which does not contain the cartridge—is inserted until it engages into a length of pressure tubing attached to the Kipps. The gas is turned on full, ignited at the outlet pipette, and allowed to blow through the apparatus for a few seconds. The hydrogen is then turned off slowly, and at the same time the tip of the outlet pipette is sealed in the bunsen flame. The inlet pipette is next withdrawn, and its tip at once sealed.

Lastly, the wide portion of the outlet pipette containing the cartridge is heated moderately in the flame. The apparatus is then ready for the incubator.

To remove the plate culture, it is necessary first to cut off the sealed end of one of the pipettes; the plate can then be easily levered from its bed with the point of a knife. The pipettes if made long enough will last for several cultures. The cultures when removed from the holders may be covered with the tin lids after sterilising them by flaming their inner surfaces.

The whole procedure of cultivation by this method is quite quick and convenient and clean, and unattended with danger *provided there is no gross leakage from defective soldering*. Before use, the plate-holder should be carefully tested for leakage, especially at the sites of attachment of the pipes. This is best done by immersing the holder in water, and blowing through each pipe in turn with the communicating holes blocked with the finger. On one occasion, a gross leakage in one of the pipes resulted in the plate being forced from its bed with explosive violence during the passage of hydrogen.

Below are given notes of culture experiments designed to test the apparatus.

COMPARISON OF SURVIVAL FROM INOCULATION IN PLATE CULTURES AND IN DEEP CULTURES.

In certain experiments carried out with a broth known as P 3, a very high degree of survival from inoculation of certain strains of anaerobes was found to occur in P 3 agar when inoculated as "shake" cultures with seekings of known numbers of organisms (de Smidt, 1923). It was decided, therefore, to test the apparatus, by means of surface plate-cultures and shake-cultures in P 3 agar inoculated in series with as much uniformity as possible; using strains which consistently gave a high yield of colonies in P 3 agar shake-cultures. Uniform inoculation of surface and deep cultures is not easily procurable. An approximation to it was found by inoculation with drops of a saline suspension of spores delivered from a capillary pipette. The pipette was marked some few inches above the end, and after flaming it, a column of the suspension was allowed to flow up to the mark. The sides of the pipette were carefully drained of excess fluid, and one drop allowed to fall upon the surface of the well-dried agar plate. Similar drops of spore suspension were let fall into tubes of uniform diameter containing 10 c.c. of melted agar at 40–45° C. In doing this, the inoculating pipette was introduced into the culture tubes held at an angle, and the drop let fall from close to the surface of the medium. Each tube was then rotated gently 25 times between the palms of the hands before allowing to set. The drop placed on the agar plate was thoroughly spread over the surface with a thin sterile glass rod bent at an angle. A variable number of organisms are lost by sticking to the spreader; in the shake-cultures only a very small proportion of organisms are lost by coming to rest in the oxygenated zone at the surface of the agar column.

Colonies in the shake-cultures were counted when the smallest present had

reached a size of about 1 mm. diameter. The suspensions of spores used were such that one capillary pipette drop yielded considerably less than 100 colonies. Such numbers of colonies are readily counted in a shake-culture tube by marking small quadrilateral areas on its surface with a grease pencil. The colonies in the plate-cultures were counted by similarly marking the surface of the Petri capsule.

The following table gives the colonies counted in plate-cultures and shake-cultures inoculated with *B. sporogenes* of different strains and *B. tetani*.

	Shake-cultures	Hydrogen plate
<i>B. sporogenes</i> ...	18-22	9
	54-67	13
	21-25	18
	27-43	<i>Confluent growth</i>
<i>B. tetani</i> ...	3-1	<i>Filmy growth</i>

In two of the above experiments, the plate was insufficiently dried for discrete colonies.

The next table shows comparisons of the hydrogen plate with a well-known single plate method depending upon alkaline pyrogallate for anaerobiosis. The plates here were inoculated with drops of very light saline suspensions of vegetative organisms from young broth cultures.

	Hydrogen plate	Pyrogallate plate
<i>B. tetani</i> ...	68	Nil
<i>Type 3 c</i> (McIntosh) ...	30	Nil
<i>B. sporogenes</i> ...	69	Nil

The hydrogen plate method has been in use for three years and has given uniformly satisfactory results. Failure of cultures when traced to the apparatus are invariably due to small defects in soldering, resulting in leakage which usually is only discovered by cultural tests.

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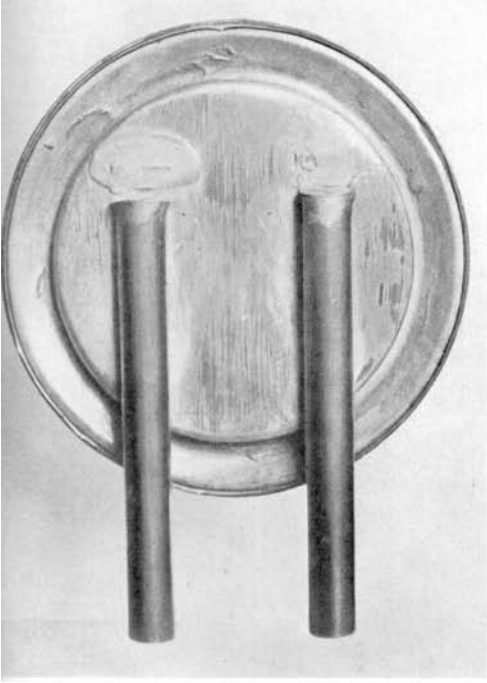


Fig. 1



Fig. 2

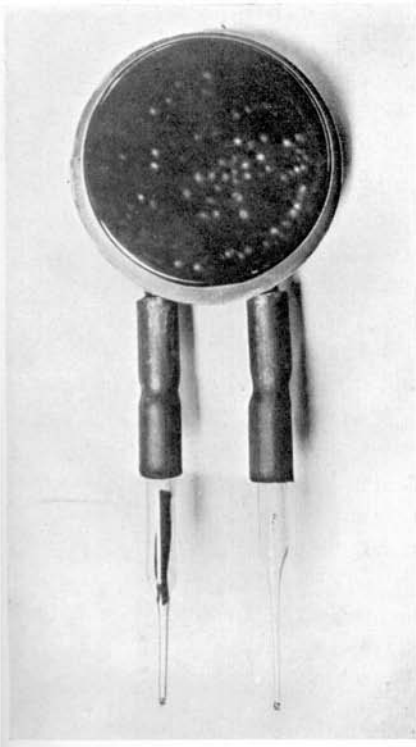


Fig. 3

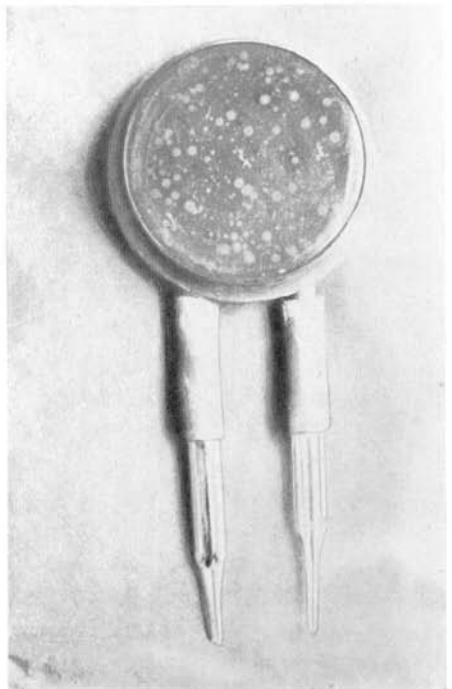


Fig. 4