A METHOD FOR CULTIVATING TREPONEMA PALLIDUM IN FLUID MEDIA.

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Treponema pallidum is an obligatory anaerobe which together with certain other spirochætæ forms a special class of organisms whose growth requires the presence of a fresh, sterile tissue in culture media.[1] The growth of *Treponema pallidum* in any suitable medium is very slow, and continues for a considerable length of time. In a solid medium, for which the pallidum shows a particular preference, the growth continues for about two months after transplantation. On the other hand, the cultivation of the pallidum in a fluid medium, with the addition of fresh tissue, by means of an anaerobic jar in which oxygen is removed through the combination of vacuum, displacement with hydrogen and absorption by pyrogallol, has given rather inconstant results. In one series of cultivations the growth may be quite favorable, while in others it may fail altogether. The loss of time and material from unsuccessful fluid cultivations is very great and the entire procedure is therefore in need of improvement.

The method which I am about to describe in detail has already proved to be entirely satisfactory and possesses certain special features that are lacking in the usual anaerobic cultural methods now in general use. In the case of the usual method, an ordinary test-tube is filled with the fluid in which the cultivation of an organism is intended, the fluid is then inoculated, and cultivated in a sealed jar under anaerobic conditions. The process is quite satisfactory for the cultivation of most anaerobic bacteria, but, as has already been mentioned, it is unreliable for obtaining growth of the

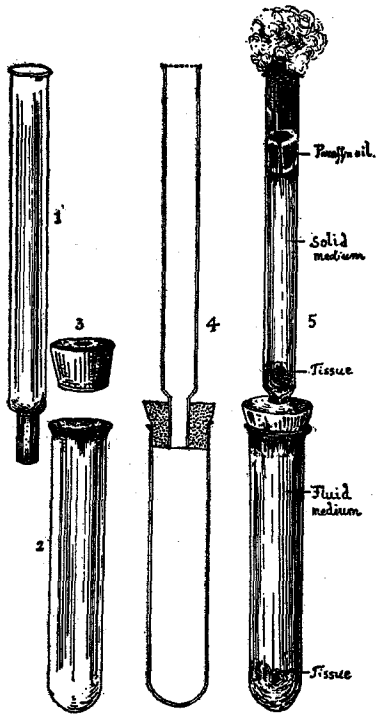
[1] It may be stated that the addition of sterile fresh tissue to a fluid medium alone is not only inadequate to make the pallidum grow, but is unable to keep it alive for any length of time. In this respect the pallidum is very different from the obligatory anaerobic bacteria, as the latter grow abundantly in such a medium.

pallidum in a fluid medium even with the addition of fresh tissue. The failure to effect cultivation of the pallidum by this method is doubtless due to the fact that with this process the removal of oxygen is often too incomplete to enable the inoculated pallidum to grow or even to survive the time required by the fresh tissue to absorb the remaining oxygen and render the fluid perfectly anaerobic. In other words, *Treponema pallidum* is too delicate an organism to survive the unfavorable conditions which last for some time within the fluid in which it is freshly transplanted. The source of this difficulty can, however, be removed by providing the organism with a more suitable medium in which it can at once begin to adjust itself. A solid medium consisting of a mixture of ascitic fluid and agar with the addition of a fresh, sterile tissue fulfills this requirement. Thus the new method which is designed to exclude this difficulty is composed of two distinct parts; one for the solid and the other for the fluid cultivation. By combining both the solid and the fluid culture media for simultaneous cultivation, the shortcoming of the latter is easily supplemented by the former which is highly suitable for the growth of the pallidum. By this method the pallidum grows at first in the solid medium as usual, and then, when the cultural conditions in the fluid portion become favorable, it migrates from the solid portion into the fluid and multiplies there abundantly.

A solid medium in this method not only serves as a reservoir of the growing spirochætæ for the fluid culture, but it is also a good indicator for determining the purity of the culture; for should any contamination occur, it is infallibly indicated by the development of the colonies of bacteria in the agar column, while it is quite difficult to detect a contamination with certain bacteria in a fluid medium alone. As will be seen later, the entire process of cultivation is carried on without the aid of a sealed jar, vacuum, or pyrogallol. The state of growth, or any changes that may take place in the medium, can easily be observed at any time without disturbing the cultivation, and, if desired, the content of the culture may, under aseptic precautions, be drawn out and examined. These are the features which may be regarded as special advantages of the new process in comparison with the older procedures.

METHOD.

Construction of the Culture Tube.—This consists of a test-tube, 1.7 centimeters wide and 20 centimeters long, whose bottom ends in a hollow projection made by fusing a short piece of a strong glass tubing, 0.7 of a centimeter bore, to the perforated bottom (text-figure, 1); a large test tube, 2.5 centimeters wide and 15 centimeters long (text-figure, 2); and a perforated rubber cork, size 5 (text-figure, 3). These parts are combined by connecting the two tubes by means of the rubber cork (text-figure, 4). The



TEXT-FIG. For description see text, p. 213.

upper tube is intended for the solid, and the lower for the fluid culture medium.

Preparations for Cultivation.—The double tube just described is thoroughly cleaned and dried; the upper tube is then plugged with non-absorbent cotton. Just before use the whole is sterilized in an

autoclave. In the meantime pieces of sterile, fresh rabbit kidney are prepared. A slightly alkaline agar (2 per cent.) is also freshly prepared, autoclaved, and kept in a fluid state. A sufficient quantity of sterile paraffin oil should be kept on hand.

Process of Cultivation.—1. After the double tube cools, one or two pieces of the sterile, fresh tissue are put into the lower tube. This is done by removing the rubber cork and the upper tube together, which are quickly replaced and fitted as tightly as possible. These parts must not be loosened again. Another piece of tissue is now put into the upper tube. The tissue goes down to the perforated bottom of the tube, but it should be large enough not to pass through the connecting hole into the lower tube.

2. After tissues have been placed in both tubes, the lower tube is filled with ascitic fluid or a mixture of ascitic fluid and bouillon. This is done by means of a large, sterile bulb pipette whose tubular portion is so drawn out as to pass through the connecting tube with a margin of space that permits the air that is displaced by the fluid to escape. The lower tube must be filled up until there is no air bubble inside the tube.

3. The inoculation of the pallidum is next made. By means of a long capillary pipette, made by drawing a sterile glass tubing, a sufficient quantity of a well growing culture is aspirated and used for inoculation of the double tube. For this purpose a syringe with a connecting pressure rubber tubing is recommended. First the capillary pipette is inserted into the fluid of the lower tube into which some of the contents are forced; then the remaining portion of the culture in the pipette is emptied into the upper tube just about the tissue.

4. When the inoculation has been performed, the upper tube is filled with a solid medium. The medium is prepared by mixing one part of ascitic fluid with two parts of slightly alkaline agar in a sterile flask. The mixing is done when the temperature of the melted agar is about 42° C. Before solidification of the ascitic agar sets in, the medium is quickly distributed into the upper tube. The quantity of the medium is about fifteen cubic centimeters.

5. The last step is to add an arbitrary amount of sterile paraffin

oil in order to cover the surface of the solid medium. About three cubic centimeters suffice (text-figure, 5).

6. The culture is then incubated at 37° C.

For the sake of clearness I have described the procedure as if I were dealing with a single set of double tubes, but in practice it is my custom to employ twelve sets at a time. The advantages of using several sets at a time are twofold. The first is an economic advantage: when one kidney is removed from a normal rabbit it usually furnishes enough tissue to fill at least six sets. As I usually sacrifice one rabbit, I have enough tissue for a dozen sets. The second advantage in making several sets is that, owing to the numerous steps that must be taken in making one series of cultivation, the possibilities of contamination are very great and it is best to provide against this accident so as to insure success.

As regards the capacity of the culture tubes, it is understood that it depends altogether upon the purpose of the work and whether or not a large quantity of the culture is desired. As the nature of my work requires as large a quantity of the material as I can obtain, I use comparatively large tubes.[2] But, according to my experience, it is not advisable to employ still larger tubes, as the result may be an enormous waste of material when accidental contamination takes place.

The method described in this article is especially adapted for the pure cultivation of *Treponema pallidum, Treponema microdentium, Treponema macrodentium; Treponema refringens, Treponema mucosum,* and *Treponema pertenue* in fluid media, when these organisms have been growing already in solid media. It is unsuitable for obtaining the growth of these spirochætæ when they are associated with other bacteria.

SUMMARY.

1. A method is described for obtaining pure cultures of *Treponema pallidum* and allied species of spirochætæ in fluid media.

2. The principle of the method is based upon the superposition of a favorable culture medium upon a less favorable one.

3. The method here described is not suited to the growth of spirochætæ when they are admixed with contaminating bacteria.

[2] The fluid culture of *Treponema pallidum* is used for the preparation of luetin as well as for immunity studies.