A CULTURE MEDIUM FOR MAINTAINING STOCK CULTURES OF THE MENINGOCOCCUS

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The maintenance of certain pathogenic bacteria upon artificial culture media is sometimes attended with great difficulty. Among these organisms the meningococcus may be said to occupy the first place. Its peculiar biology—particularly its intra-cellular ferment which is so potent a factor in its destruction—makes its viability at all times precarious. Furthermore its highly parasitic nature requires highly complex substances such as those upon which it grows in the human body.

While strains of the meningococcus that have been accustomed to artificial cultivation may be maintained upon plain nutrient agar, this medium is not favorable to its continued cultivation; the addition of glycerine offers no advantage; glucose results in more rapid growth and consequently more rapid degeneration. In their early work Councilman, Mallory and Wright used Loeffler's blood serum for both isolation and maintenance. Flexner used plain agar to which sheep serum was added. Some authors have used the serum of other animals—horse, goat, calf. Human serum and human ascitic fluid are conceded to be superior for isolation and for obtaining massive growth. The addition of the whole blood is possibly better than serum alone. Kutscher recommends a medium prepared with human placenta to which is added calf serum and glucose. For isolation Conradi used the centrifugalized spinal fluid, adding one part of the supernatant liquid to three parts of slightly alkaline nutrient agar; upon this solidified medium he planted the sediment.

Fluid media offer no advantage over solid media and they are of course not adaptable for isolation. Gelatin is not suitable because the meningococcus does not grow at low temperatures.

While many of these media, offer satisfactory conditions for growth during a few generations, a fair proportion of strains kept upon them suddenly fail to develop and in spite of persistent effort cannot be resuscitated. Furthermore on all the above mentioned media the cultures under ordinary conditions must be transplanted at short intervals—not longer than two or three days—and kept constantly in the incubator at 37.5°C. (Exceptional strains are found which are unusually hardy and seem to require little more care than the common saprophytic bacteria.)

With all the above culture media, those favoring the rapid growth of the meningococcus at the same time result in rapid ferment production and consequently rapid death of the culture.

After innumerable trials we have found a medium which permits of relatively slow growth of the meningococcus with apparent suppression of ferment formation, thus resulting in greater viability. This medium has been in use for about two years and its superiority over the other media mentioned in the literature for the maintenance of stock cultures of the meningococcus seems to warrant its publication. This culture medium is a modification of the potato-blood-agar used by Bordet and Gengou for the isolation of *B. pertussis*.

*Preparation of medium*

1. Prepare potato extract as follows:
   a. Potato peeled, cut in small pieces and washed in running water for about two hours, 100 grams.
   b. Water containing 4 per cent double distilled glycerine free from acid, 200 cc.
   c. Mix and autoclave for forty minutes.
   d. Allow to stand over night and strain through cheese cloth.
2. Make potato-extract-agar as follows:
   a. Mix in an Erlenmeyer flask: Potato extract, 50 cc.; NaCl solution 0.65 per cent, 150 cc.; agar 5 grams.
b. Heat in Arnold sterilizer until agar is melted, requiring from thirty minutes to one hour.

3. Tube without filtering, and sterilize, in autoclave for about forty minutes.

4. When wanted for use, melt the agar, cool to about 45°C. and add the desired amount of sterile defibrinated horse blood.

The amount of blood to be added depends upon whether or not the meningococcus has become accustomed to the medium.

In transplanting from another medium to this potato blood agar, a little difficulty may be experienced in getting the cultures started upon the new substratum. For this reason a large amount of the growth (not over twenty-four hours old) should be transferred to the medium containing about 20 per cent of defibrinated blood. In making the inoculation the culture should be rubbed slightly into the surface. This is incubated at 37.5°C. for about 2 days and then transplanted again to the potato-extract-blood-agar containing just sufficient blood to permit growth—that is, about 5 per cent. Subsequent transplants need not be made more often than every thirteen to fifteen days or longer, when kept at 37°C., provided that the cultures do not become too dry. In the case of cultures paraffined or sealed to prevent drying, a fair growth may be obtained after six weeks.

The meningococcus grows well at 37.5°C. At lower temperatures it will remain alive for a considerable length of time, although no growth occurs; viability may be retained at room temperature apparently as long as, if not longer than, at incubation temperature. Besides, maintenance at this temperature renders paraffining or sealing less imperative for the prevention of drying. Freshly transplanted cultures that were incubated for twenty-four hours at 37.5°C., and then kept at room temperature, showed fair growth after 4 weeks. Ice box temperature will kill most strains of the meningococcus in a comparatively short time. Cultures grown at 37.5°C. for twenty-four hours, and then transferred to the ice box, grew well after five days, but after ten days about twenty-five per cent failed to show any growth at all, 50 per cent showed scanty growth, and only 25 per cent a fair growth.
The meningococcus is an aerobic organism but like many other aerobes when first grown aerobically and then transferred to an atmosphere of hydrogen, it can be kept alive longer than when oxygen is present. Cultures of meningococci were grown aerobically for twenty-four hours at 37.5°C and then transferred to a Novy jar, the air of which was replaced with hydrogen by means of a Kipp apparatus and a Schutte vacuum pump; the jar contained pyrogallic acid and sodium hydroxide which were permitted to mix after the air had been replaced several times by hydrogen. These cultures were then kept in the incubator; after ten weeks good growths were obtained on the first transplant.

The appearance of the meningococcus growth on potato blood agar is not very characteristic. After twenty-four hours growth at 37.5°C the individual colony has reached the size of a small pinhead. It is gray in color, smooth and rather moist looking, of an amorphous consistency, the surface elevation varying from convex to pulvinate with border entire. With age, the color of the colony changes to dull gray, the consistency becomes tenacious, and the surface elevation more of the raised type.

Although the production of pigment by some organisms is facilitated on potato blood agar, the area of discoloration characteristic of numerous strains of streptococci,—notably the *Streptococcus viridans* and pneumococcus on blood agar, and some Gram negative cocci on glucose agar as described by Elser and Huntoon—has never been observed by us with any strain.

All of our thirty-eight strains have invariably remained Gram negative, regardless of culture medium used or age of culture. Occasionally, more frequently in old cultures, a few organisms may be seen that do resist for a time the action of the decolorizing agent and thus appear to be Gram positive. However, this is usually an indication of faulty technique or of contamination.

Arrangement in pairs is most common, although single cocci and groupings in tetrads are numerous, with certain strains especially. True chain formation has never been observed. Variations in the size of organisms in the different strains are
negligible, those of individual cocci seeming to be determined more by the age of the culture, than by the culture medium. Degeneration forms occur with all strains in quite young cultures.

The potato-blood-agar furthermore is of value for differentiating between the meningococcus and the gonococcus; on this medium the gonococcus grows only when the medium has a high blood and diminished salt content—the growth is always very scanty and the characteristic differences are immediately apparent.