A MODIFICATION OF ROSENTHAL'S CHROMIUM-SULFURIC ACID METHOD FOR ANAEROBIC CULTURES

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In planning a detailed study of the growth requirements of *Clostridium tetani*, it became necessary to fix on a suitable method of anaerobiosis. Since it was anticipated that the investigation might well extend over a considerable period of time, there were several criteria which it seemed ought to be fulfilled. For example, the method selected should lead to rapid, complete and reproducible anaerobic conditions, should not be cumbersome, should be safe, and finally, of course, should be as economical as possible.

The available methods, now more or less standard, include the hydrogen-CO_2-palladium jar of McIntosh and Fildes, the very simple sprouting oats jar, the phosphorus method, and the recent chromium-sulfuric acid method of Rosenthal. The first was rejected as being relatively time-consuming and not altogether without danger, although in other respects admirable. The oat jar is slow in becoming anaerobic, frequently requiring 24 hours for decolorization of the methylene blue indicator tube. The routine use of phosphorus is too dangerous. This leaves the chromium method, with the single disadvantage that it provides no CO_2 and would consequently be unsuitable for rapid growth. This shortcoming was easily remedied by the addition of Na_2CO_3 to the chromium powder, and experiment showed that prompt growth from small inocula of tetanus bacilli took place under these

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conditions, whereas the lag period was tremendously prolonged when no CO_2 was provided.

Calculation showed that the amounts of both chromium and sulfuric acid recommended by Rosenthal were in excess of the actual requirements, and as a matter of economy these may be considerably reduced. Extended experiment has shown that in the type of jar described below, 1.5 grams chromium powder, 0.5 gram Na_2CO_3 and 15 ml. of 15 per cent H_2SO_4 (by volume) per liter capacity can be depended upon to decolorize methylene blue promptly in glucose bouillon, and keep it decolorized as long as the jar is tightly sealed.

It was proposed to carry out the nutrition work in fluid medium in ordinary 6-inch tubes. A 2-quart Ball fruit jar suggested itself as the most economical container suitable for the purpose. Such a jar will hold about 25 ordinary 6 x $\frac{5}{8}$″ culture tubes, which are readily inserted and removed with long forceps over whose tips short lengths of rubber tubing have been drawn. Brass castings were made at negligible cost, using the glass jar caps as patterns. A single hole is drilled through the cap, into which is soldered a short length of brass tubing. This is attached to a short glass U tube readily made from 5 or 7 mm. glass tubing. The open end of the latter dips below the surface of about 2.0 ml. of mercury in the bottom of a Wasserman tube cut off about 2 inches long, and this in turn is secured to the brass tube by a rubber band. A small plug of cotton helps avoid the spattering of the mercury. An ordinary fruit jar rubber ring is used, which is moistened with glycerol before use, helping to secure an air-tight joint.

In use, 3 grams of chromium powder and 1 gram of Na_2CO_3 are put in the jar, the cultures and an indicator tube consisting of a loop-ful of Loeffler's methylene blue in a tube of glucose broth are put in place, and 30 ml. of 15 per cent sulfuric acid are added through a funnel in order to avoid wetting the stoppers. The cap is clamped on without unnecessary delay. Hydrogen and CO_2 are evolved, the excess gas escapes through the mercury trap, and the reaction is practically complete in 5 minutes. The jar can then be placed in the incubator without further attention.

SUMMARY

A slight modification of Rosenthal's chromium method is presented which provides the CO_2 essential to rapid growth of anaerobes and effects some economy in operation. A simple and inexpensive type of jar suitable for anaerobic test-tube cultures is described.

REFERENCE

ROSENTHAL, L. 1937 "Chromium-sulfuric acid" method for anaerobic cultures. J. Bact., **34**, 317–320.