ON THE AMOUNT OF CARBON DIOXIDE SUPPLIED FOR THE PRIMARY ISOLATION OF NEISSERIA GONORRHOEAE

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The importance of increased carbon dioxide tension for the primary isolation of *Neisseria gonorrhoeae* is well recognized. Some of the methods advocated for increasing the carbon dioxide in the atmosphere in which the cultures are to be incubated are (1) replacing about 10 per cent of the air in the container with carbon dioxide from a tank, (2) mixing measured amounts of sodium bicarbonate solution and dilute sulfuric acid so as to obtain the desired volume of carbon dioxide, (3) placing a lighted, smokeless candle in the container with the cultures before closing the container, and (4) placing a handful of moistened oats in the container with the cultures before closing the container.

To our knowledge and experience, all of these methods are satisfactory for supplying the necessary carbon dioxide in the environment in which the cultures are to be incubated. The first method cited supplies more carbon dioxide than is necessary. The work of Ferguson (1945) supports that conclusion as he found that strains of the gonococcus grew "equally well on 'chocolate' agar in a zone of carbon dioxide enrichment with a lower limit between 2 and 2.3 per cent and an upper limit between 18 and 22 per cent." With the second method cited it likewise is possible to supply any desired volume of carbon dioxide by using the proper amounts of sodium bicarbonate and sulfuric acid, but the use of liquids is not always the most practical method.

The placing of a lighted, smokeless candle in the container with the cultures before closing the container is a satisfactory method for supplying the necessary carbon dioxide, but there appears to be a discrepancy in the amount of carbon dioxide which is produced. Nye and Lamb (1936) reported that about 3 per cent is the maximum amount of carbon dioxide produced in a jar by burning a candle. On the other hand, Carpenter (1943) stated, "When the flame is extinguished by the depletion of the oxygen, the concentration of carbon dioxide is approximately 10 per cent." When we were making a series of primary isolations of the gonococcus (Morton and Leberman, 1944), we tested the air within some of the jars after the candle had become extinguished and found that the volume of carbon dioxide ranged from 2.0 to 2.5 per cent. Recently, Ferguson (1945) reported that the jars contained about 2.3 per cent after burning a candle in a closed container. Our results and those of Ferguson and of Nye and Lamb are in agreement.

While investigating the amount of carbon dioxide produced by burning a candle, we also tested the amount of carbon dioxide produced in a closed container containing germinating oats. After 48 hours' incubation at about 36 C

the air in a closed container holding cultures and germinating oats, as recommended by Christensen and Schoenlein (1940), contained approximately 5 per cent carbon dioxide.

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