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A Differential Plating Medium for Lipase-Producing Bacteria.*

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A need for a differential plating medium for lipase-producing bacteria was felt when a study was begun a year and a half ago for the purpose of classifying the bacterial flora of the gastro-intestinal canal in certain diseases possibly due to bacteria present there; *i. e.*, pellagra, sprue and pernicious anemia. Since diet has such an important bearing upon these diseases, it was decided to try as a basis for classification, in part at least, the action of the various bacteria present on certain fundamental elements of food, *i. e.*, fats, starch, protein. This seems practicable only by means of differential plating media.

The medium under discussion has been in use for more than a year and only a partial study of its possibilities has been made. However, since it might be useful to others, it was thought well to publish a description at this time. The composition of the media is as follows: Sugar free meat digest fluid, 1,000 cc.; Di-basic sodium phosphate, 5 gm.; Agar, 30 gm. Melt, clear, adjust for final pH of 7.6. Autoclave. Add 0.125 gm. nile blue sulphate in 100 cc. 25% ethyl alcohol. Tube in 6 to 7 cc. amounts in tubes already sterile. Arnold for 15 minutes. Store. To use, the agar is melted and cooled to 45°-50° C. A sterile emulsion of fat is added to about 10% volume; 0.7 cc. for 7 cc. tube. We use either cream or one of cotton seed oil made according to directions in U. S. Pharmacopeia for codliver oil except 25% more water is used than prescribed. The

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inoculum and emulsion are thoroughly mixed with agar, poured into a petri dish, and allowed to harden. Incubation has been in the anaerobic jar, the air of which has been exhausted by burning phosphorus. Twenty-four hours is usually sufficient to bring out the typical colony. Surface streaked plates are not so satisfactory as plates that are inoculated and then poured, since the surface colony does not always give the characteristic picture.

The lipase-producing colony is identified by the dark blue zone surrounding it and is in striking contrast to the dull bluish gray color of the remainder of the plate. The colony itself is usually colorless. The zones are usually of greater diameter than the colonies except in those colonies with marked tendency to spread. When examined with the low power microscope, the zone is seen to be made up of tiny blue globules or shapeless masses of the same color. None of the colorless globules of oil seen elsewhere through the plate is observed in the zone. If the colony under study is well separated from others, a faded circular zone 2-3 mm. wide will be observed surrounding the blue zone. (This is probably due to diffusion of dye toward the colony to take the place of that taken up by the fatty acids and soaps.)

In studies on gastro-intestinal contents of some 15 patients, 10 strains of bacteria producing the deep blue zone have been isolated in pure culture. When these were grown in sugar-free meat infusion broth plus cotton seed oil emulsion, evidences of digestion of fat were noted. One strain, which has since been lost, would, in 2 or 3 days, change a layer of emulsion 3 mm. thick into a fluffy layer 8 mm. thick. After this change the material would stain deep blue with nile blue sulphate, which it would not do before. As to the other strains, the results are not so striking, but gross evidences of fat splitting are obvious. From cultures showing either marked or slight gross change in the fat layer, fatty acids or soap may be obtained by extracting with ether from an alkaline solution, then precipitation by hydrochloric acid.

No organism giving the deep blue zone described has been found without marked lipolytic action. However, 3 organisms have been isolated which gave a transient purple zone and which in pure culture in liquid media produced no lipase. On removal of the plates from the anaerobic jar, these would show only a faded area about the colony. After standing an hour, a pale purple haze develops, the colony being unstained. This color is localized to the tiny globules. It lasts only 2 to 3 hours.

As is well known, certain bacteria, especially anaerobes, possess the power of permanently decolorizing practically any of the analine

dyes. If colonies of this type are closely placed on the plate, the lipase producing colony will not be detected. However, if the colonies are spaced as much as 3 or 4 mm. apart, it is not interfered with. The plate may be crowded with thousands of colonies of weak or no reducing power, without interfering with the production of the typical blue zone.

A possible source of error in considering a colony as a lipase producer, which it is not, lies in the fact that certain bacterial colonies themselves will take the stain. It is then necessary to distinguish between a blue colony and a blue zone, which is usually not difficult.

ERRATUM—Page 21—*B. enteritides* should read *B. **enteritidis***.

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