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A Simple Culture Medium for Endamoeba Histolytica.

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As contrasted with various media generally used in the cultivation of *Endamoeba histolytica,* the proposed medium is simple in its composition. It consists of sterile nutrient broth and a mixture of starch and charcoal. The nutrient broth is adjusted to pH 7.0 and is left in an incubator until used, or warmed to about body temperature at the time of inoculation of a suspected material. To the above medium is added rice starch and animal charcoal mixture (in the proportion of 2:1 by volume) briefly designated as S.C. mixture in this paper. A small amount of the S.C. mixture is loosely placed in a small tube and sterilized by dry heat at 180°C. for 45 minutes. Rice starch, according to Dobell and Laidlaw,[1] provides the amoebae with a definite source of assimilable carbohydrates and also inhibits the growth of *Blastocystis* frequently found in feces. Animal charcoal is used to adsorb ammonia and hydrogen sulphide present in the culture, thus reducing their deleterious effects upon the amoebae, while calcium phosphate contained therein helps to stimulate the metabolic activities of the organisms.

Formed stool is thoroughly mixed by means of a sterile glass rod in order to obtain a uniform distribution of the cysts. Liquid stool

[1] Dobell, C., and Laidlaw, P. P., *Parasitol.*, 1926, **18**, 283.

should first be sedimented, and the supernatant fluid decanted. Washed cysts are then prepared by filtration and sedimentation of the stool, followed by repeated washings with centrifuging. Cysts in freshly sedimented stools grow almost as well as those obtained in the stools left standing for several hours at room temperature or in a refrigerator.

Procedure. A small amount of washed cysts (0.1 cc. or less dependent upon the number of cysts present) is introduced into 8 cc. of the broth, thoroughly mixed by shaking and a 5 mm. loopful of the S.C. mixture is added. The tube is now incubated at 37°C., the optimum incubation period being 24-48 hours. By means of a 1 cc. pipette with a wide terminal opening, the sediment is at first gently scraped off from the bottom of the tube, and then about 0.1 cc. is withdrawn. This is spread on a warm clean slide, covered with a cover glass, and examined by means of a 10 X eye piece and 16 mm. dry objective preferably either on a warm stage or in a warm chamber. The addition of a small drop of 0.1% neutral red will facilitate the examination greatly, as it gives the amoebae a pinkish refractive tinge as contrasted with other objects in the microscopic fields. Care should be taken to guard against draught at any stage of the procedure, as this may cause the amoebae to round up and die.

By the use of this medium, the amoebae are often seen at various stages of development. The medium also seems to be practically a specific one for *E. histolytica,* as other endamoebae showed none or only an extremely poor growth in it. The rate of growth of the amoebae in this medium is very striking. Thus, by the introduction of 80 cysts, the number of trophozoites was calculated to be 21,200 at 48 hours incubation in one instance, while in the others, 40 cysts gave rise to 17,900 and 72 cysts to 12,200 trophozoites.

Furthermore, this medium may be employed very effectively by covering the coagulated egg slant of Boeck and Drbohlav,[2] or that of Dorset.[3]

Immunological studies are in progress with this culture material as an antigen.

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[2] Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, **5**, 371.

[3] Dorset, M., *Am. Med.*, 1902, **3**, 555.