conditions described. In view of these facts the work indeed has seemed very encouraging.

Recently we obtained leprosy nodules through the kindness of Dr. O. E. Denney at Carville, La., and with the chick embryo tissue method have attempted to isolate Hansen's bacillus from these fresh cases. If our original organism was the authentic leprosy bacillus it should be possible to cultivate, in tissue medium, the true leprosy germ from fresh lepromata. We had nodules from 3 different cases, emulsions of which were all contaminated with non-acid-fast organisms when received. Such emulsions also contained a vast number of acid-fast organisms, presumably Hansen's bacillus. These emulsions we have treated and concentrated with 3% sodium hydroxide to destroy contaminants and have succeeded in cultivating the acid-fast organism from each of these 3 cases in young chick embryo tissue suspended in Tyrode's solution as we have the older Puerto Rican strain. Isolation and growth of acid-fasts from fresh human leprosy tissue seems to be as easily accomplished as the continued growth of our older strain. We believe this presents new and convincing evidence that in these cultivation studies we have without doubt been dealing with the actual causative agent of leprosy, if Hansen's bacillus is to be accepted as the cause of this disease. These strains of acid-fasts which we are able to cultivate from leprosy lesions do not grow on any artificial mediums, in so far as we have tested the several ordinary laboratory media, under ordinary atmospheric conditions. Only in the tissue medium does actual multiplication take place under ordinary atmospheric conditions and it is presumed that in such tissue media we have a CO_2 tension similar to that obtained under the artificial conditions employed by us, as well as other elements which favor multiplication of the microbe under study.

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Egg-Oyster Media for the Cultivation of Acid-Fast Bacteria.

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Since oysters have been shown to be a good source of vitamins A, B, C, and D as well as to contain in considerable amounts the inorganic elements iron, copper, manganese, zinc, lead, arsenic and

iodine,[1] the possibility of the use of oysters in culture mediums was suggested. It was found that many organisms, including the group of acid-fast bacteria, grow quite luxuriantly on certain of the mediums prepared. Two solid egg-oyster mediums, one made with the yolks and the other with the whites, and a hormone oyster infusion broth have proven to be the most satisfactory of all preparations.

Six strains of *Mycobacterium tuberculosis* (H 37, R_1, a virulent human strain obtained from Dr. Novy, a virulent bovine strain, a virulent avian strain and an avirulent strain) and 9 strains of chromogenic acid-fast bacteria isolated from lepers by several investigators all give typical growth on both the egg white and egg yolk oyster mediums. The strain of *M. leprae* isolated by McKinley and Soule[2, 3, 4] has been successfully cultivated on the egg white oyster when incubated in an atmosphere of 10% carbon dioxide and 40% oxygen for 30 days, but does not appear to multiply on the egg yolk oyster medium when incubated under similar conditions. The value of these mediums for the isolation of acid-fast organisms is being studied further.

The egg oyster mediums are prepared by mixing (a) 2 parts of egg white with 1½ parts of minced oyster; or (b) 1½ parts of egg yolk, ½ part of whole egg and 2 parts of minced oyster. The pH of each mixture is adjusted to about 8.2. The tubes are placed in an inspissator at a temperature of 70 to 80°C. immediately upon filling so that the tissue does not settle to the bottom of the tubes before coagulation. After coagulation the temperature is brought to between 80 and 90°C. and sterilization is carried out at this temperature for one hour on each of 4 successive days. The oyster hormone infusion broth is made as Huntoon's[5] hormone medium broth with the substitution of an equal amount of minced oyster for the ground beef heart and the omission of the laked blood.

[1] Levine, H., Remington, R. E., and Culp, F. B., *J. Nutrition*, 1931, **4**, 469.

[2] McKinley, Earl B., and Soule, Malcolm H., *J. Am. Med. Assn.*, 1932, **98**, 361.

[3] Soule, Malcolm H., and McKinley, Earl B., *Am. J. Trop. Med.*, 1932, **12**, 1.

[4] Soule, Malcolm H., and McKinley, Earl B., *Am. J. Trop. Med.*, 1932, **12**, 441.

[5] Levine, M., and Schoenlein, H. W., A Compilation of Culture Media, Williams and Wilkins, Baltimore, 1930.