blood vessels, as in typical rickets. Part of these rats were fed the Steenbock and Black[1] diet No. 2965. Cod liver oil added to this diet in amounts varying from 2 per cent of the diet up to a quantity where food became objectionable, did not prevent the development of rickets in these rats. Other thyroidectomized rats of the same age were fed a diet consisting of table scraps of the various kinds of food constituting the dietary of well-nourished individuals. These rats also developed rickets but in a somewhat lesser degree. Unthyroidectomized control rats of the same litter, living on identical dietaries, did not develop rickets, as determined by histological examination of the epiphysis of the long bones. These experiments demonstrate the necessity of the thyroid hormone for the complete utilization of cod liver oil.

This is a preliminary report.

[1] Steenbock and Black, *J. Biol. Chem.*, 1925, lxiv, 263-298.

3497

Microbic Dissociation: The Tubercle Bacillus.

S. A. PETROFF.

From the Research and Clinical Laboratory of Trudeau Foundation, Trudeau, N. Y.

The studies of dissociation of R and S colonies in various types of microorganisms suggested an investigation of a similar nature with the tubercle bacillus. Here we encounter many difficulties. Workers with tubercle bacilli are aware of the fact that it is almost impossible to prepare a suspension free of clumps and that there are very seldom individual organisms in the suspension. Secondly, as the organisms develop very slowly, sometimes requiring four weeks for a visible colony to appear, contaminations are liable to occur and spoil the investigations of even the most careful workers; and thirdly, it is almost impossible sometimes to prevent dehydration of the culture medium upon which the organism is cultivated. When the tubercle bacilli are cultivated on media prepared in Petri dishes contamination occurs in a large number of the plates. These contaminations are not due to the initial exposure during the seeding, but to the leakage of the air within the plates. In the incubator temperature the air is expanded and again contracted when the Petri dishes are removed to room temperature where the air rushes into the plates, carrying along the contaminating molds and other organisms.

In the following study we have used for subculturing the tubercle bacilli the author's gentian-violet-egg medium made up in Petri dishes. (The procedure for preparation of the medium and inspissation can be found in any text book.) The suspension of the organism was prepared as follows: Individual colonies were removed from the original culture and triturated with platinum wire on the inside wall of a small tube containing 2 cc. of sterile salt solution with a pH of 7.8. After trituration was completed, the tubes were rotated until all of the masses of the organism were dispersed in the salt solution. This was then filtered through two layers of sterile filter paper and the filtrate was inoculated on the surface of the medium mentioned above. To prevent evaporation and contamination we have adapted the following method which proved very satisfactory: Rubber bands 3½ inches long and 1½ inches wide were slipped over the edge of the Petri dish so that each half of the Petri dish was covered with ¾ inch of the rubber band, resulting in a good tight fitting.

Up to the present, two types of tubercle bacilli have been investigated. The B 1 strain is a tubercle bacillus of bovine origin of moderate virulence for rabbits, which have been in our possession

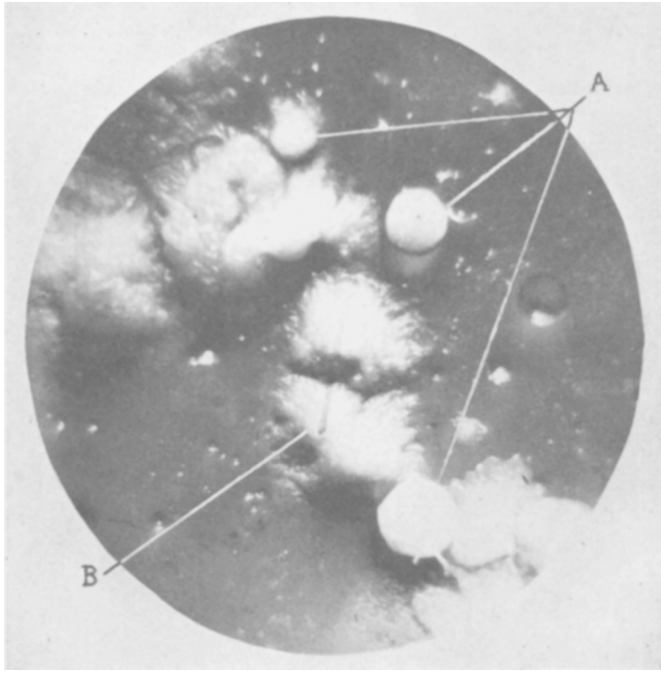

FIG. 1.

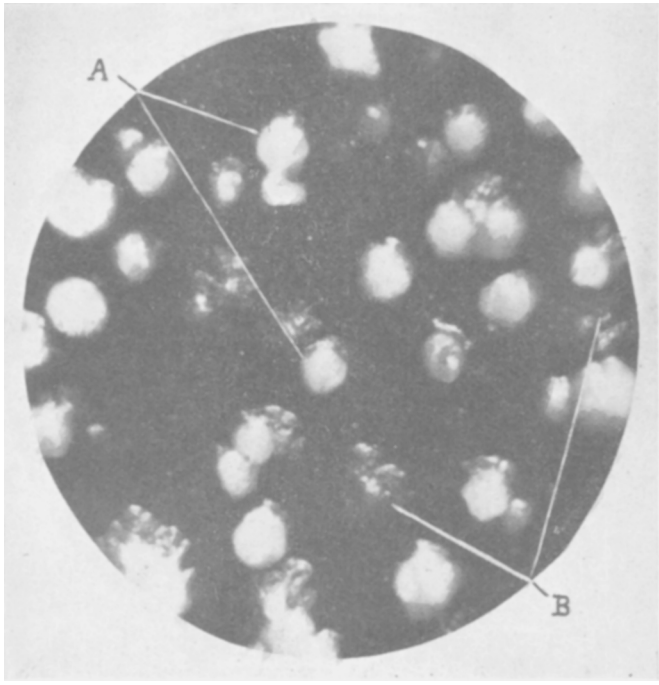

FIG. 2.

for a number of years. In the photograph it can be clearly seen that there are two distinct types or colonies, one (Fig. 1 A) being perfectly round, smooth, glistening and resembling small moth balls, while the second type of colony (Fig. 1 B) is very rough, irregular and dry, spreading right into the culture media. The second organism is of avian type, which was very kindly sent to me by Dr. Moore of Ithaca. Here, again, it is very evident from the picture that one (Fig. 2 B) of the colonies is almost transparent, rather irregular and spreading, while the other (Fig. 2 A) is more of the granular opaque type, very much raised and smooth. The former is moist, while the latter is dry.

At present work is in progress to study various phenomena which have been observed with R and S colonies with the other organisms. It seems that the tubercle bacillus in all probability does not vary in regard to "dissociation" from that seen in the other types of micro-organisms.

This is a preliminary report.