REPRINTED ARTICLE

CULTIVATION OF *Mycobacterium Leprae*. III[1]

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The experiments here recorded were made with the object of confirming in a distant land and with patients of another race our earlier studies [(1)] on the isolation and cultivation of the leprosy bacillus. A description was given of a slow growing non-chromogenic acid-fast organism isolated from human leprous tissue by incubating the inoculated media in an atmosphere of 40% O_2 and 10% CO_2. There was unequivocal evidence of the proliferation of the germs but it was also true that the quantity was limited. The organisms were removed from the positive tubes and transferred to freshly prepared sterile media; after an incubation period of about 6 weeks the tiny colonies that had developed were subcultured as before. A total of 26 series subcultures were made, but there was no apparent improvement on the part of the cells to assume a saprophytic existence. The culture in the 26th generation showed the same characteristics as at the beginning of the series.

A subsequent communication [(2)] reported efforts to provide a more suitable substratum. Several of the complex formulae ordinarily employed for the isolation of such species as Johne's bacillus and the tubercle bacillus were prepared and inoculated; in addition amino acids and fresh vegetable tissues were incorporated in several of the usual laboratory media. The results were uniformly disappointing. The overwhelming numbers of organisms in the lesions of the disease and the impoverished growth in test tubes led to the conclusion that some essential food factor was lacking in the nutritive media.

There have been several criticisms directed at the studies carried out in Puerto Rico; the most important of which has been the suggestion, that in making the serial subcultures, tissue debris containing stainable organisms was mechanically transferred from tube to tube, leading to the erroneous conclusion that proliferation had occurred when actually no *in vitro* multiplication had taken place. The appearance of definite colonies, although of limited size gave assurance that such was not the case; however in planning the protocols for the studies in the Philippines adequate controls were included to obviate this as well as other objections.

The details of the experiments followed in general the technique used in Puerto Rico. Clinical specimens (excised nodule emulsions, subepidermal pus from "lepra reaction" cases and broken down nodules) rich in acid-fast forms were transferred to the surfaces of

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the media contained in tubes; usually 6 of these were inoculated from each specimen. The tubes were then placed in Novy jars and a special gaseous environment was provided containing 10% CO_2 and 40% O_2. After an incubation period of 4-6 weeks the media were examined macroscopically for the presence of minute colonies and microscopically for acid-fast forms. In every cultural attempt to control the mere mechanical transfer of stainable cells, there were placed in the jar tubes which were inoculated with a droplet of the original specimen that had been autoclaved to insure the death of the cells. This method of killing the organisms did not alter their tinctorial properties. When the control tubes were examined microscopically it was possible to find after a careful search a few well formed acid-fast rods in the subcultures up to and including the 6th serial transfer, but not thereafter.

The tubes considered positive at the end of the incubation period were subcultured to similar media, placed in the artificial gaseous environment and the incubation again carried out. The data are presented in Table 1.

TABLE 1.—*Isolation of slow growing acid-fast organisms, supposedly Hansen's bacillus, from various types of infected material.*

Origin of specimen	Total no. of specimens inoculated	No. of specimens giving positive cultures
Nodules	20	12
Reaction pus	16	11
Broken down nodules	6	2
Total	42	25

The colonies appearing on the various media were identical with those previously described, and in each instance the organisms were acid-fast. No other colonial or morphological types were observed in the cultures.

The encouraging results reported by McKinley and Verder [3] in the cultivation of the leprosy bacillus with the aid of minced chick embryo tissue suggested the desirability of similar studies at Culion. Accordingly, the minced tissue suspensions were prepared in Erlenmeyer flasks and subsequently inoculated with fresh infected human tissue rich in acid-fast forms. Twenty-six specimens were studied following the technique given by these workers with the addition of controls inoculated with autoclaved material. In 22 instances there was unquestioned evidence of proliferation.[2] It was possible to find stainable cells in control flasks but the numbers were negligible. The positive cultures were serially subcultured for 6 transfers in the tissue-containing medium without any marked improvement as regards multi-

[2]McKinley and Verder had noted early in their work the similarity of the rod shaped pigment cells of the chick retina with clumps of bacilli and had kindly issued the warning not to be misled by these forms.

plication. At various times, test tubes containing the media of Petragnani or Löwenstein were inoculated with organisms from the tissue culture flasks with the hope that the bacteria might possibly be acclimatized to a saprophytic existence after this long residence away from the human host and grow luxuriantly, but such was not the case. The colonies appearing on the special media developed very slowly and were of the typical tiny variety.

The writer cultivated the germ of leprosy from infected tissue according to the original technique of Carrel. The results have been uniformly disappointing in that growth, though positive, was no more luxuriant than that obtained with macerated chick embryo tissue.

The isolation and serial cultivation of a slow-growing non-chromogenic acid-fast organism from human leprosy tissue has been confirmed. The limited multiplication of the germs indicated that the ideal media and environment for their saprophytic existence has not been provided.

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