CULTIVATION OF LEPROSY BACILLI

AND OF THE TUBERCLE BACILLUS FROM LEPROSY TISSUES

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INTRODUCTION

Since Hansen's discovery of the leprosy bacillus efforts to cultivate it and to reproduce the disease experimentally have been made continuously by investigators in the various countries. Nevertheless, as Klingmüller says (3), these objectives cannot be accepted as having been achieved.

Since April, 1929, we have attempted this cultivation using nodules from 43 patients on 45 occasions, a lymph gland from 1 patient, and blood from 73 patients. The first acid-fast bacillus which we cultivated proved to be the tubercle bacillus. Subsequently, however, we have obtained from 14 patients 15 strains of organisms that we consider to be the leprosy bacillus (14, 15, 18). We have made animal

inoculations and complement-fixation and intracutaneous reactions with many of these strains, and have concluded that they are *Mycobacterium leprae.*

LEPROSY CULTURE WORK IN JAPAN

Before describing our experiments we wish to summarize the attempts made in Japan to cultivate this bacillus. Noteworthy reports have come recently from elsewhere, one by Henderson in Calcutta, and another by McKinley and Soule from Puerto Rico[1]—but these are accessible. On the other hand little of the Japanese work is known abroad.

According to Nagayo's list (10) the first report appears to be Shibayama's, in 1899 (22). He repeatedly cultivated from leprous pus and intact lesions a bacillus which formed transparent colonies in 24 hours from pus, or in 7 to 9 days from other materials, and that was extremely pleomorphic (diphtheroid), almost completely Gram negative, and partly decolorized by Ziehl-Gabbet. It cannot be regarded as the leprosy bacillus. In the same year Murata and Kino (9) also cultivated from leprous tissue a bacillus that was not resistant to alcohol and which they considered a diphtheroid.

Nakano (11) in 1911 transplanted leprous material to the abdominal cavity of the rabbit and found marked proliferation of bacilli several days after the death of the animal. After transplanting the same material into the corpses of guinea pigs, or to their spleens and livers, at room temperature, he always found proliferation of the bacilli, but they were always mixed with other bacteria.

One of us (Ota), in 1915-1916 used the method recommended by Emile-Weil, Reenstierna, Kedrowsky, Puschtiwoi, and Teich, but obtained only marked proliferation of bacilli in a leprous suspension preserved in bouillon. Harada (1) obtained similar proliferation in various kinds of bouillon with korikonnyaku[2], and Y. Hayashi in Ringer's solution (1918), but this cannot be considered successful cultivation.

In recent years reports by Shiga and his colleagues and by Nojima have stimulated active work on this problem in Japan. Shiga (23) in 1928 observed the proliferation of leprosy bacilli on potato, and Shiga, Matsuoka and Kobashi (24), showed that the bacilli are aerobic and not destroyed by 5 per cent sulphuric acid, and that glycerine, egg, asparagin, seaweed, phosphate, potato and unpolished rice powder are of value for their growth. Their results, together with those of Keil and Unna (1927) and Schlossmann (1930), have furnished much information. However, the bacilli usually formed no visible colonies. The strain that did finally produce a colony grew slowly and showed dry wrinkles; its cuti-reaction in leprosy patients paralleled that to tubercle toxin; it caused cer-

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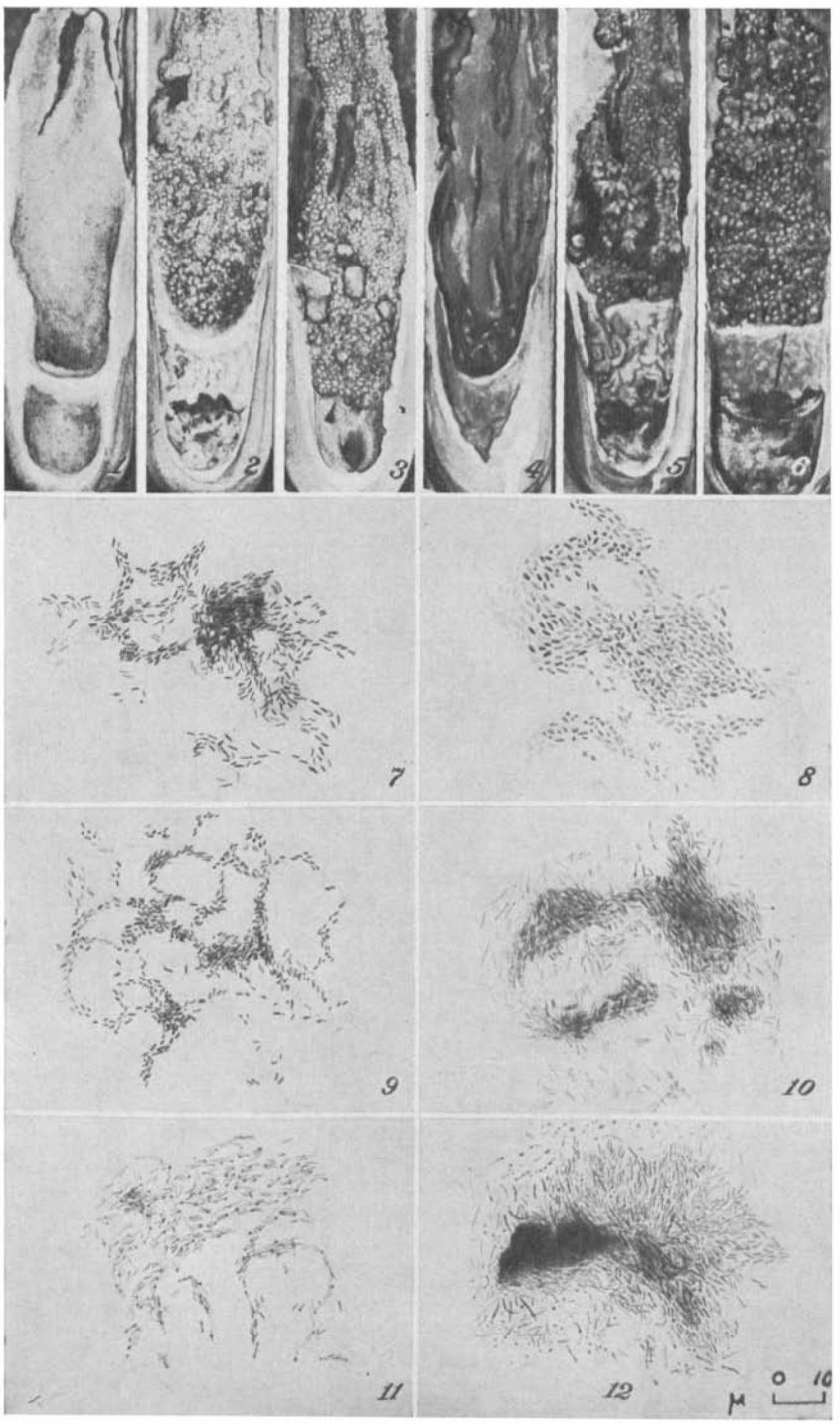

PLATE 1.