

6 SKIN TESTING WITH LEPROSY BACILLUS SUSPENSIONS

TO THE EDITOR:

As requested, I am supplying a free translation of the summary of a report which I made at the April 1918 session of the Japanese Bacteriological Association on attempts which I had made to cultivate the leprosy bacillus, and of the results of skin tests made on leprosy patients with the supposed cultures which

had been obtained. This report appeared, under the title, "On a Pure Culture of Leprosy Bacilli, and a Skin Reaction by Means of the Pure Culture Suspension," in the *Saikingaku Zasshi* (*Journal of Bacteriology*) No. 272 (1918) 51-53, published by the Kitasato Institute. The entire summary is given, because the part dealing with the cultivation work explains the material used in the skin tests.

*On a pure culture of leprosy bacilli.*—Since 1914 I have been investigating methods of obtaining pure cultures of the leprosy bacillus, but have not yet found a perfect method. However, I have obtained relatively marked reproduction of the bacilli *in vitro* by means of the following method: (a) Ringer's solution is placed in test tubes, 10 cc. per tube, and sterilized. This is used as the culture medium. (b) A new nodule is extirpated entire, and many tiny slices are made of it. One such slice is placed in a tube of the culture medium, and the tubes are incubated at 34° to 37°C. The entire procedure is carried out with care to avoid contamination with other bacteria.

In this way, by 3 to 5 weeks we can observe a slightly yellowish and viscous material manifesting gradual multiplication around the slice at the bottom of the test tube. The upper part of the medium is almost transparent. If the tube is shaken the whole medium becomes turbid, of a thin milky appearance. A smear of this suspension reveals large numbers of leprosy bacilli in aggregates which are much larger than what can be found in the original nodule. Furthermore, the numbers of bacilli observed in such smears are far greater than those in a preparation made from an equivalent quantity of a suspension of a nodule slice of the same kind and same size. No colony formation can be seen in transplants of the culture upon normal agar or glycerine agar media.

From these facts it is believed that the numerous bacilli in the Ringer's solution signify reproduction and isolation from the original nodule slice. It is therefore concluded that leprosy bacilli are able to reproduce *in vitro*.

2. *On a skin reaction in leprosy.*—I tried a skin-reaction test by means of an antigen prepared in the following manner: The culture suspension of leprosy bacilli described above was heated at 60°C. for 30 minutes, an equal quantity of sterile Ringer's solution was added, and finally 0.5 per cent of carbolic. For a control, sterile Ringer's solution was used.

In the first step, 0.1 cc. of the antigen was inoculated into the abdominal skin of five healthy guinea-pigs. This resulted in absolutely negative reactions.

In the next step, 60 leprosy patients, of which 44 were of the lepromatous type and 16 were neural-type cases, were tested. The antigen was inoculated, 0.1 cc., into the skin of the flexor surface of the upper arm. The results were as follows: Twelve of the 44 lepromatous-type cases (27.3%) were positive, and 32 (72.7%) were negative. Twelve of the 16 neural type cases (75%) were positive, and 4 (25%) were negative.

According to these results the neural type gives a high rate of positive reactions, while the lepromatous type gives few positive and many negative reactions. This is a very interesting result which calls for attention.

Practically the same results were obtained by means of a suspension

of nodule, except that the reactions were always stronger than those caused by the culture suspension, especially in the case of the neural-type patients.

I had not tried this skin-reaction test on normal healthy people, and therefore cannot discuss the diagnostic value of the test in leprosy.

My idea when the work with the skin test was undertaken was that lepromatous cases should give strongly positive reactions and neural-type cases weakly positive reactions, and that nonleprous people should be negative. The results were completely opposite to that assumption, and I was much interested in that fact.

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