CORRESPONDENCE

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CULTIVATION OF MYCOBACTERIUM LEPRAE

To the EDITOR:

As one of the many workers in leprosy who have been puzzled by the published results of recent attempts to cultivate *Mycobacterium leprae,* I am writing to suggest that those who are in a position to do so might, through the medium of your JOURNAL, throw a little light on the subject.

We have for several years been trying to grow this organism in vitro, and also to verify the claims of successful cultivations made by other workers, but have met with complete failure. Among the many methods tried exhaustively have been those advocated by Shiga, Ota and Sato, Soule and McKinley, and McKinley and Verder. Recently we have been trying to get the organism to grow in tissue cultures, a method for which Salle and others have claimed success.

I would particularly mention the confusion and doubt which seems to surround the work of Soule, McKinley and Verder. Soule and McKinley in 1932, and McKinley and Verder in 1933, published several papers in which they claimed success: (a) by keeping the cultures in an atmosphere of 40 per cent oxygen and 10 per cent carbon dioxide (Soule and McKinley), and (b) by the use of chick embryo minced in Tyrode solution as a medium (McKinley and Verder). Two of these articles were reprinted in the JOURNAL [1 (1933) 53 and 351]. The claims made by these workers were very definite. McKinley and Verder reported obtaining multiplication of *My. leprae* in five days, and marked growth in ten days, in minced chick embryo medium. After many attempts we have completely failed to get any multiplication of the organism by these methods. Dr. N. E. Wayson, recently the director of the leprosy investigation station in Honolulu, informs me that he has had exactly the same experience. It was, therefore, with some interest that I read in the *Journal of the American Medical Association* [104 (1935) 285] the following statement

quoted from an article by McKinley on the etiology of leprosy published in *Medicine* [13 (1934) 377]:

> In fact it must be stated today, sixty years after Hansen first saw *Mycobacterium leprae,* that there exists no absolute proof as yet that any investigator during all these years has actually succeeded in cultivating *Mycobacterium leprae* in vitro.

Unfortunately, the publication in which that appeared is not available in India, so I have not been able to look up the context of this quotation. McKinley has been part author of five articles in which successful culture of *My. leprae* has been claimed. Does this mean that he has now changed his mind regarding the genuineness of the cultures obtained by him and his co-workers? The present situation is confusing.

Regarding the work of Salle published in the JOURNAL [2 (1934) 201], our attempts to repeat this work have failed. It is possible that the organism multiplies in tissue cultures but we find that this multiplication, if it occurs at all, is very slow, and we have completely failed to observe the nonacid-fast diphtheroid forms which Salle has reported.

It is noticeable that most of the publications regarding culture of *My. leprae* claim success, while many negative results go unreported. May I suggest that negative results in attempts at culture are valuable, sometimes much more so than the reports of positive results, that it would be worth while for workers to publish in the JOURNAL brief reports of their attempts to culture the organism of leprosy?

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Comment by Dr. Earl B. McKinley, Washington, D. C.

The courtesy of an opportunity to comment on the letter of Dr. Lowe regarding the cultivation of *Mycobacterium leprae* permits me to clarify our position on this subject and, I hope, to do away with some of the confusion to which he refers.

The original paper of the writer with Soule [*Jour. American Med. Assoc.* 98 (1932) 361-367] was read at the meeting of the American Medical Association held in Philadelphia in June, 1931. During the same month our second paper appeared [*American Jour. Trop. Med.* 12 (1932) 1-36], and later in the year a further paper with Soule appeared in the same periodical (pages 441-452). Soule has since published a report of independent work in the Philippines [*Proc. Soc. Exper. Biol. and Med.* 31 (1934) 1197-1199]. Meanwhile the writer with Verder published preliminary studies on methods of cultivation in tissue culture [*Proc. Soc. Exp. Biol. and Med.* 30 (1933) 659-616 and 31 (1933) 295-296]. Finally,

in December, 1934, the writer published a monograph on the etiology of leprosy [*Medicine* 13 (1934) 377-504]. It is probable that a statement made in that publication, a statement in which an effort was made to be utterly conservative and fair in the discussion of this question, is largely responsible for the confusion to which Lowe has referred.

Let me say at the beginning that we have no thought of changing our minds regarding the genuineness of our cultures. We believe them to be the true germ of leprosy. This has been our belief from the time of our first report and still is in 1935, four years later. I have learned from various sources that some leprosy workers are under the impression that in my monograph I have withdrawn the claim with regard to our cultures. In this they are mistaken.

It is true that I made the following statement quoted by Lowe. I would emphasize this part of it: "*...that there exists no absolute proof as yet...*" This is not a retraction of our former work. It is simply an honest and fair statement indicating that in this problem we realize our inability to fulfill Koch's postulates to our entire satisfaction and to that of other investigators. Nevertheless, we still believe our organism to be most significant.

Referring again to my monograph, the discussion from which Lowe's excerpt is taken is continued as follows:

> We are well aware that there are those investigators who will not be willing to agree with this statement, probably feeling that the organisms cultivated by them from the tissues of lepers represent the true *Mycobacterium leprae*. We can appreciate this point of view. Yet the author with his colleagues, who have also advanced cultures which they feel are probably *Mycobacterium leprae*, are of the opinion that this is the only fair statement which can be made at this time in the matter of cultivation of the leprosy bacillus. We feel definitely that we have an organism which has more in its favor than any other organism which has been submitted as *Mycobacterium leprae*. We feel that we have perhaps gone somewhat further in establishing this organism as *Mycobacterium leprae* through animal experimentation. Yet the organism we isolate from leprosy tissue is grown with only great difficulty and is very sparse in its growth, and we have not succeeded in producing in laboratory animals the counterpart of leprosy in man.

And further on (page 480) it is said:

> As for animal experimentation, we feel again that the only fair statement which can be made at the present time is that no investigator has to date succeeded in producing the counterpart of human leprosy in any experimental animal. Naturally we include our own attempts in this direction in this statement.

From the foregoing I believe it will be entirely clear just what we mean. We feel that we have definitely cultivated the true germ of leprosy. We cannot prove it. We know that in time other investigators using the same methods will isolate this organism. We have accomplished this and have obtained living cultures with definite colony formation—with only few colonies, it is true, even after four years since isolation. Because this organism grows with difficulty, though the technique of culturing is not complicated, and since growth is no better after four years of isolation, we feel this organism fits the picture of the theoretical germ of leprosy better than any other which has been reported in culture. Therefore, we are led to believe that it is the true *My. leprae*. Time and future experiment will give the final answer, and we are content to await confirmation of the results we have reported to date, being certain of the outcome.

In reference to the tissue cultures, here again we feel that we have definite multiplication of this acid-fast organism, because we have carried it from tissue culture to solid media with definite colony formation. We have not met with the nonacid-fast forms reported by Salle except in a few instances, and these diphtheroids we regard purely as secondary invaders of devitalized tissue and of no importance with regard to the *My. leprae.*

Lowe writes that he failed to confirm our work on cultivation in chick embryo and says that Dr. Wayson had informed him that he has had exactly the same experience. Let me call attention to a statement published in the Annual Report of the Surgeon General of the United States Public Health Service for 1932, page 30, in regard to the use of chick embryo tissue for the cultivation of *My. leprae,* which reads in part: "...in three instances of the cultures of human material there has apparently been a proliferation of the acid-fast bacilli planted and a definite growth of a diphtheroid in from five to seven days after inoculation." This report is indicated as coming from Wayson's laboratory. It continues with the information that all of these cultures were carried through several transplants, one of them through fifteen transplants. The acid-fasts in the last transplants seemed to be as numerous as those in the original culture. Naturally, I am at a loss to understand the comments of Lowe concerning the views of Wayson in reference to the above.

Lowe's letter is most timely, and I am glad to have an opportunity to present this explanation to appear with his communication. I feel that any discussion of cultivation of *My. leprae* may possibly stimulate additional investigation, which is much needed.