The purpose of this note is to call the attention of the present generation of leprosy workers to the work of Malcolm H. Soule on the cultivation of the leprosy bacillus. The results of that work, the first phase of which was done in association with Earl B. McKinley a full thirty years ago, seem to have been forgotten. They were, admittedly, controversial, and there is no record of anybody having succeeded in repeating them. Nevertheless, the writer—who personally observed the second phase of it, and made a later follow-up—is convinced that they were real, and wishes to go on record to that effect. A non-chromogenic bacillus of a kind not reported before, transplantable but very slow and sparse in growth and often difficult to maintain in sub-cultures, was repeatedly cultivated from lepromatous leprosy lesions.

McKinley, who in 1928 was assigned by the College of Physicians and Surgeons of Columbia University to the directorship of the School of Tropical Medicine in Puerto Rico, arranged in 1930 with Soule, who at the University of Michigan had started out as a chemist but soon turned bacteriologist, to join him for a year as a visiting professor.

Together they attacked the problem of the cultivation of the leprosy bacillus. In 1932[1,2,3] they reported success in cultivating, in a partial-tension atmosphere (40% O_2 and 10% CO_2), a bacillus with the characteristics described. Long afterward McKinley[4] told how difficult

1. SOULE, M. H. and MCKINLEY, E. B. Cultivation of B. leprae with experimental lesions in monkeys. American J. Trop. Med. **12** (1932) 1-36.
2. SOULE, M. H. and MCKINLEY, E. B. Further studies on experimental leprosy and cultivation of Mycobacterium leprae. American J. Trop. Med. **12** (1932) 441-452.
3. MCKINLEY, E. B. and SOULE, M. H. Studies on leprosy. Experimental lesions in monkeys and cultivation of *Bacillus leprae*. J. American Med. Assoc. **98** (1932) 361-367.
4. MCKINLEY, E. B. The bacteriology of leprosy; a review. Internat. J. Leprosy **7** (1939) 1-28, 217-255.

it was to maintain this microorganism in subculture; most of the strains had failed to grow in one or another of the subcultures. The positive "tubes" (presumably meaning strains) were only 6 in the 13th generation, 5 in the 14th, 3 in the 15th, and 2 in the 16th.

At the time, too, they believed that they had also infected monkeys, but the lesions produced were of tuberculoid histology, presumably of the nature of the reactions to lepromin.

It had long been an intriguing puzzle why so many people in various parts of the world had recovered from leprosy so many strains of acidfasts, most of them chromogenic and all apparently different. Nobody reporting success in cultivation ever had an opportunity to repeat his work in another part of the world, with patients of another race in a different environment. And so in 1933 (we being medical director of the Leonard Wood Memorial at the time), arrangements were made for Soule to come to Culion for several months to repeat his work here. In the meantime McKinley, then dean of the George Washington Medical School in Washington, D.C., had pursued, with Verder, his study of the germ isolated in Puerto Rico.[5,6] For one thing, the bacilli were said to have been cultivated—still sparsely—in a liquid medium containing minced chicken embryos.

At Culion, using the original technic employed in Puerto Rico with "hormone" agar, Soule succeeded in obtaining 25 positive cultures from 42 specimens of lepromatous lesions—12 from 20 ordinary nodules, 2 from 6 broken-down nodules, and 11 from 16 specimens of pus from lepra reaction cases. No other kinds of microorganisms appeared in the cultures, neither diphtheroids nor chromogenic acidfasts. The cultures grown appeared to be identical with those isolated in Puerto Rico. By none of the various measures employed could better growths be obtained—better adaptation to saprophytic life. This work being done in our laboratory, we observed it personally.

To meet an objection raised in connection with the Puerto Rico work, i.e., that the bacilli in the cultures were merely carryovers from those in the tissues of the original inocula—although the formation of minute colonies in the subcultures should have answered that—Soule controlled each inoculation by autoclaving a portion of seed material used. The killed bacilli disappeared entirely after a few transfers.

Soule published a brief preliminary announcement of this work in a periodical that few leprologists see,[7] although it was later discussed in other articles to be cited; a promised full report for THE JOURNAL did not materialize. That report ended with the following statement:

> The isolation and serial cultivation of a slow-growing non-chromogenic acid-fast

5. McKINLEY, E. B. and VERDER, E. Cultivation of Mycobacterium leprae. Proc. Soc. Exper. Biol. & Med. **30** (1933) 659-661.
6. McKINLEY, E. B. and VERDER, E. Further studies on cultivation of *Mycobacterium leprae*. Proc. Soc. Exper. Biol. & Med. **31** (1933) 295-296.
7. SOULE, M. H. Cultivation of Mycobacterium leprae. III. Proc. Soc. Exper. Biol. & Med. **31** (1934) 1197-1199.

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.

Lowe,[8] in correspondence, said that he had failed completely to confirm the results reported by Soule and McKinley. He was also confused by a statement by McKinley[9] that:

". . . there exists no positive proof as yet that any investigator . . . has actually succeeded in cultivating *Mycobacterium leprae* in vitro."

In reply, McKinley said that they still believed that their cultures were the true germ of leprosy. To explain the statement cited he quoted from the next paragraph of the publication cited by Lowe (which Lowe had not seen):

". . . Yet the author with his colleagues, who have . . . 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. . . . 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*. . . . Yet the organism we isolate from leprosy tissue is grown only with great difficulty and is very sparse in its growth. . ."

In June of 1937, in an A.A.A.S. symposium,[10] Soule and McKinley reported that at that time 2 of the Puerto Rico strains had been maintained through 40 serial generations over a period of 6 years. Of the Culion strains, 2 had been maintained for 4 years and were then in their 18th generation. The symposium discussion of this work ended with a citation of the conclusion in Soule's 1934 report, with the added statement that "This is our belief today."

At the Cairo Congress in 1938 Soule read a paper on the subject, the only record of which is an abstract[11] in which precisely the same thing is said. The majority report of the Bacteriology Committee of that Congress, of which Soule was a member, said that the problems of the *in vitro* growth of the leprosy bacillus "have not yet been solved satisfactorily."[12] This is in accord with previous statements by both Soule and McKinley, who had emphasized the qualifying word "satisfactorily."

In his extensive review of the bacteriology of leprosy, McKinley[4] reviewed both phases of the culture work in detail, and in summary was most conservative. He did not claim that cultivation of *M. leprae* had been accomplished; he only said that in this work ". . . we have perhaps the most promising advances yet reported." As evidence in favor of that possibility, he pointed out the facts that the germ was different from any ordinarily reported, that it was apparently ex-

8. Lowe, J. Cultivation of Mycobacterium leprae. Internat. J. Leprosy **4** (1936) 234-235 (*correspondence*).
9. McKinley, E. B. The etiology of leprosy. Medicine **13** (1934) 377-504.
10. Soule, M. H. and McKinley, E. B. The bacteriology and immunology of leprosy. *In* Tuberculosis and Leprosy, the Mycobacterial Diseases, Symposium Series Vol. I, American Assoc. Adv. Sci. Lancaster, Pa., Science Press Printing Co., 1938, pp. 87-96.
11. Soule, M. H. The cultivation of Hansen's bacillus. Internat. J. Leprosy **6** (1938) 465-466 (abstract).
12. [Cairo Congress] Report of the Subcommittee on *in vitro* cultivation of *M. leprae*. Internat. J. Leprosy **6** (1938) 408.

tremely delicate of constitution and was difficult to isolate or maintain, and that it was nonchromogenic. "These findings" he said, "should be tested thoroughly and rigidly by other investigators." This has not been done, to our knowledge.

Only one worker, so far as we know, confirmed Soule's findings. In Singapore in 1936 it was learned that Professor Young, of the Medical School of the University of Malaya, had done so and he was interviewed. Whether or not he demonstrated cultures is, unhappily, not remembered. Known to his colleagues as a notorious nonreporter, he evidently wrote nothing on the subject.

After the Cairo Congress (1938) we visited Soule at Ann Arbor for the follow-up mentioned. At that time he was carrying on only two selected strains, one isolated in Puerto Rico seven years before, and the other a Culion strain then five years old. The growth habits of neither strain had changed during the years; the tubes showed only very slight growths, best seen in reflected light, as had the Culion strains when first isolated. A smear of each strain showed abundant acid-fast bacilli. In one of the smears the bacilli were arranged in strands, which were within and surrounded by an unstained substance, clearly outlined against the bluish background. This brought to mind the matrix substance (gloea) of globi.

In general, skepticism about this matter has prevailed. It has not been forgotten, however, and it is known that new investigations, repeating the old are in prospect. That should be done by investigators in a position to do so. Personally, as said, we are convinced of the validity of the results reported by Soule and McKinley, and of the modest claims they made regarding them. That avenue of approach has been ignored much too long.

—H. W. Wade