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CULTIVATION OF MYCOBACTERIUM LEPRAE

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McKinley and Soule [1] reported the successful cultivation of *Mycobacterium leprae*, obtained from Puerto Rican lepers, on several culture media. Subsequent reports were made by Soule and McKinley, [2, 3] when their nonchromogenic strain of acid-fast bacilli, believed to be the true *Mycobacterium leprae*, had been carried through the eighth and sixteenth generations respectively, the latter representing cultivation over a period of 18 months. Experimental protocols were also presented dealing with suggestive experimental lesions produced in 2 species of monkeys. In the cultivation work it was apparent that the leprosy microbe was maintained on artificial media with greater difficulty with each generation or transfer. In the sixteenth generation, so-called, after the organism had been on artificial media for some 18 months, only 2 definitely positive cultures resulted. One of these cultures has been employed in an attempt to ascertain better methods of cultivation.

A logical procedure was to attempt cultivation in embryonic tissue. Minced chick embryo 7 to 11 days old was washed and suspended in Tyrode's solution. Human embryonic tissue has also been employed, but with less success. In the chick embryo, suspension, growth of the original *Mycobacterium leprae* has been stimulated. Growth is obtained within 5 days under CO_2 and O tension as well as under ordinary atmospheric conditions in the incubator. Several additional generations or transfers have been added to the 16 previously reported with this organism. Growth of *Mycobacterium leprae* has also been obtained with human embryonic spleen tissue suspended in Tyrode's solution, but this tissue is more difficult to

[1] McKinley, Earl B., and Soule, Malcolm H., *J. Am. Med. Assn.*, (1932) 98, 361.

[2] Soule, Malcolm H., and McKinley, Earl B., *Am. J. Trop. Med.*, (1932) 12, 1.

[3] Soule, Malcolm H., and McKinley, Earl B., *Am. J. Trop. Med.*, (1932) 12, 441.

obtain and is no longer employed in our routine culture work. With the young chick embryo tissue medium we are now able to cultivate, apparently indefinitely, this strain of *Mycobacterium leprae.*

There has always been doubt, when claims have been made for the cultivation of the true causative agent of leprosy, that actual cultivation *in fact* has been accomplished. None of the methods seemed certain and many different varieties of organisms have been isolated from human leprosy materials. The organism isolated from lepers by one of us with Soule, however, has differed remarkably in its habits and the conditions necessary for growth and multiplication. Furthermore, suggestive lesions have been obtained with it in experimental animals and the organism, with all the difficulties encountered in maintaining it on artificial media, has remained viable for nearly 2 years, surviving *only* under the special gaseous conditions described. In view of these facts the work indeed has seemed very encouraging.

Recently we obtained leprosy nodules through the kindness of Dr. O. E. Denney at Carville, La., and with the chick embryo tissue method have attempted to isolate Hansen's bacillus from these fresh cases. If our original organism was the authentic leprosy bacillus it should be possible to cultivate, in tissue medium, the true leprosy germ from fresh lepromata. We had nodules from 3 different cases, emulsions of which were all contaminated with non-acid-fast organisms when received. Such emulsions also contained a vast number of acid-fast organisms, presumably Hansen's bacillus. These emulsions we have treated and concentrated with 3 per cent sodium hydroxide to destroy contaminants and have succeeded in cultivating the acid-fast organism from each of these 3 cases in young chick embryo tissue suspended in Tyrode's solution as we have the older Puerto Rican strain. Isolation and growth of acid-fasts from fresh human leprosy tissue seems to be as easily accomplished as the continued growth of our older strain. We believe this presents new and convincing evidence that in these cultivation studies we have without doubt been dealing with the actual causative agent of leprosy, if Hansen's bacillus is to be accepted as the cause of this disease. These strains of acid-fasts which we are able to cultivate from leprosy lesions do not grow on any artificial mediums, in so far as we have tested the several ordinary laboratory media, under ordinary atmospheric conditions. Only in the tissue medium does actual multiplication take place under ordinary atmospheric conditions and it is presumed

that in such tissue media we have a CO_2 tension similar to that obtained under the artificial conditions employed by us, as well as other elements which favor multiplication of the microbe under study.

ADDENDUM TO THE PRECEDING ARTICLE

Since the foregoing preliminary report was published the authors have had more experience with the use of human embryonic tissue as medium for the cultivation of *M. leprae* and *B. tuberculosis*. The latest work on the use of tissue mediums for the cultivation of these acid-fast organisms was reported in Washington, D. C., at the annual meeting of the American Association of Pathologists and Bacteriologists on May 9. At this meeting the authors reported that human embryonic spleen, liver and lung tissue has been found very satisfactory for the cultivation of both organisms mentioned; that the human tissues appear to contain something very beneficial to the growth of these organisms. In further work with the chick embryo and human embryo tissue mediums the authors find that the incubation period for these two acid-fast organisms ranges between one and two weeks, with an average incubation period of ten days. The method of preparing the tissue medium was described. It consists of washing and mincing the embryonic tissues and placing them in Tyrode's solution contained in small Erlenmeyer flasks. Five cc. of Tyrode's solution are used, and it was emphasized that only a small amount of tissue be used. In some cases 3 per cent glycerol or embryonic extract is added to the Tyrode solution. The glycerol appears to favor growth of the leprosy bacillus but not that of *B. tuberculosis* in any appreciable way. It was also reported at this meeting that the strain of *M. leprae* first reported by McKinley and Soule has now been carried through twenty-six generations over a period of over two years.[4]

[4] An article more fully descriptive of this new method of cultivating acid-fast bacilli and of results obtained in applying it to the leprosy bacillus will appear in an early issue of the JOURNAL.—EDITOR.