seat of processes of degeneration followed by repair, may as a result of the latter process revert back to a type of structure normal for a remote ancestral form; (2) the possibility of inducing an ingrowth of vessels into the kidney through the cortex in an attempt to maintain function in a relatively aglomerular structure.

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Further Studies on the Cultivation of Mycobacterium Leprae.

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We[1] have previously reported the cultivation of *Mycobacterium leprae* in minced chick embryo suspended in Tyrode's solution direct from leprous nodules containing acid-fast organisms. Evidence of growth and multiplication was obtained in as few as 5 days although subsequent experience has shown that better growth occurs in from 10 days to 2 weeks. The leprosy nodules were digested with 3% sodium hydroxide to free the organisms from the tissue and to destroy contaminants and, following neutralization, the tissue medium was inoculated. Human embryonic tissue (spleen, liver, lung) has also been employed and more experience with this tissue leads us to believe that it is a better medium than chick embryo, contrary to our previous report, although it is, of course, more difficult to obtain.

Leprosy nodules which have been treated as described above, the acid-fast organisms from which have been seeded in minced embryonic tissue suspended in Tyrode's solution, have been found to contain acid-fast organisms which are unquestionably alive. After carrying such cultures in the tissue medium through several generations we wished to test the viability of these acid-fast organisms by placing them on a solid medium, such as the hormone glycerol agar, under a gaseous tension of CO_2 and oxygen. Such cultures were prepared and were incubated under these conditions for 3 months. In one series of 10 cultures of this type we have found 6 to be positive as judged by definite micro-colonies of acid-fast organisms (quite similar to those which have been described for *B. tuberculosis*

[1] McKinley, E. B., and Verder, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 659.

obtained from the blood stream) present in smears from the cultures when such smears are taken from suspicious areas which can be identified grossly. These micro-colonies are somewhat difficult to demonstrate and require a perseverance not usually required in the ordinary casual examination of a bacteriological smear, but their identification is definite and, we believe, represents evidence that the acid-fast organisms in the tissue culture are viable after several generations in such mediums. The demonstration of these micro-colonies may prove to be significant in offering an explanation of the frequent failure to demonstrate acid-fast organisms in the very early lesions of leprosy.

Further study of the chick embryo tissue method of cultivation of the acid-fast organisms from leprosy tissue indicates that multiplication does not continue to improve with each subculture. The medium, as has been reported, tends to become quite alkaline and, while the organisms appear to grow better in the beginning they later slow up and this may be due to the reaction of the medium. The same is apparently true with *B. tuberculosis* bovine, somewhat less so with human *B. tuberculosis,* though avian *B. tuberculosis* continues to grow luxuriantly.

The method of growing various strains of acid-fast organisms in minced embryonic tissue suspended in Tyrode's solution (or in ordinary broth which has given good results in a limited number of trials) deserves further study. It is possible that such cultures might be decidedly improved if they were placed in a gaseous tension as described by one of us with Soule.[2, 3, 4] We feel that this method may eventually be employed under such favorable conditions as to give most promising results with the group of acid-fast organisms.

[2] McKinley, E. B., and Soule, M. H., *J. Am. Med. Assn.*, 1932, **98**, 361.

[3] Soule, M. H., and McKinley, E. B., *Am. J. Trop. Med.*, 1932, **12**, 1.

[4] Soule, M. H., and McKinley, E. B., *Am. J. Trop. Med.*, 1932, **12**, 441.