CORRESPONDENCE

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CULTIVATION OF *M. leprae* BY SOULE

TO THE EDITOR:

The opinion has recently been expressed in THE JOURNAL that M. H. Soule and E. B. McKinley discovered conditions essential to the cultivation of *M. leprae*.[1] I wish to enter a contrary opinion based upon the experience of others and upon my own experiments.

In 1939, when I went to Culion, I had heard of six unsuccessful attempts to confirm this work. Subsequently I repeated the experiments of Soule and McKinley, also with negative results. My experience at that time prompts the following comments.

The Huntoon medium, which Soule used in his experiments, differs from most meat infusions in that the lipids are not filtered from the extractives. These lipids tend to creep up to the surface of agars and to combine with those liberated from the inoculated tissue emulsions, so that the two coalesce in tiny droplets. Even with "cleaner" infusions the formation of "pseudocolonies," which simulate those of pleuro-pneumonia-like organisms, was of concern to Brown *et al.* (*J. Bact.* **40** (1940) 857-864). Incubation of agar slants in desiccators for controlling gaseous atmospheres permits the drying of agar surfaces at such a slow rate that pseudocolony formation develops to maximal degree. When mycobacteria are present in an inoculum containing tissue suspension, they, being lipophilic, are swept into the lipid aggregations. Since autolysis eliminates background materials which stain with methylene blue, and mycobacteria in fact are found in very great concentrations, microscopic samples seem to represent pure cultures of the latter.

That these phenomena explain the findings of Soule and McKinley was demonstrated in a three-rack experiment that I carried out at Culion. In each of the three racks there were 40 tubes of Huntoon medium inoculated with a 5 per cent emulsion of a fresh human leproma. After the stipulated incubation of coded tubes in desiccator jars enclosing 10 per cent carbon dioxide and 40 per cent oxygen, i.e., the length of incubation and gaseous concentrations used by Soule, the tubes were arranged in a long row and classified as "positive" or "negative" by careful inspection. When the positives had been uncoded and placed in the original racks, approximately equal numbers of positives were found in each rack. The experimental design was:

[1] WADE, H. W. The cultivation of *M. leprae* by Malcolm Soule. Internat. J. Leprosy **32** (1964) 201-204 (*editorial*).

Rack 1, Huntoon's medium as prescribed; Rack 2, the same plus 0.5 per cent phenol; Rack 3, the basal medium plus 1 per cent by volume of formalin solution.

I submit, therefore, that the positive findings were due to physical factors and not to microbial growth.

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