female rats ranging from 3 to 6 months in age. Infected animals and animals having irregular oestrus cycles or cycles over 6 days in length were excluded.

Animals of the same strain were kept under the conditions and on the diet described previously.[5, 6] Vaginal smears were made at intervals of 12 hours or, in most cases, of 8 hours, and oestrus was defined as the period at which an abundance of cornified cells was obtained, after the preceding smear had shown nucleated epithelial cells. Dioestrus was defined as 48 or more hours after this time. Three animals with very regular cycles were killed 20 hours before the expected onset of the next oestrus. The animals were killed with chloroform and the pituitary, thyroid and adrenal were quickly removed and weighed in a closed weighing bottle to 0.1 mg. The data on the adrenal have been previously reported.[6] The pituitary weights are thought to be accurate to about 0.2 mg. since the gland need not be dissected from surrounding tissue. The thyroid weights are much less so because of the difficulty of accurate and rapid dissection. They also include the parathyroids.

The mean actual and relative weight of the pituitary and the probable error of this mean were calculated for each group of animals. The resulting figures indicate definite and significant increase in the weight of the pituitary during oestrus, with gradual and progressive decrease during dioestrus. This decrease in animals killed 52-72 hours after oestrus amounted to approximately 20% of the relative weight at dioestrus.

The thyroid showed no significant change in weight, but the wide variation in thyroid weight in the entire series is sufficient to obscure any except an extreme change.

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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

[5] Andersen, D. H., *J. Physiol.,* 1932, **74**, 49.

[6] Andersen, D. H., and Kennedy, Helen S., *J. Physiol.,* 1932, **74**, 247.

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

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 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

[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.

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% 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.

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Egg-Oyster Media for the Cultivation of Acid-Fast Bacteria.

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Since oysters have been shown to be a good source of vitamins A, B, C, and D as well as to contain in considerable amounts the inorganic elements iron, copper, manganese, zinc, lead, arsenic and