The pulpar cells showed all the signs of degeneration including complete necrosis with karyorrhexis and pyknosis. The nuclei of the megakaryocytes also showed pyknosis. Iron pigment was absent. In the spleen also, the dissimilarity of the histological picture to that of infectious anemia, with all the signs of splenic hyperactivity, is marked.

Reviewing the changes which take place in the liver and spleen of animals dying from nutritional anemia or killed in the later stage of the disease, we note (1) the absence of any signs of hematopoiesis or hematocataresis, and (2) the presence of severe atrophic disturbances of the cells, associated with signs of failing circulation (chronic heart failure).

7258 P

Use of Living Chick Embryos in the Propagation of B. leprae.

R. A. HOLT. (Introduced by C. W. Duval.)

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Because of our failure to obtain *in vitro* multiplication of *B. leprae* in minced chick embryo media,[1] it occurred to us that living chick embryos might afford a more suitable nutritive since the studies of Duval[2] indicate that *B. leprae* is unable to cleave whole protein and that the split products (amino acids) are essential for its multiplication. This hypothesis is substantiated by the observations that the bacilli apparently enter and colonize within the living host cells without destroying them and, as intracellular sojourners, utilize the nutrients intended for the latter; that artificial culture media containing the end products of protein digestion afford a favorable foodstuff for the initial cultivation of *B. leprae;* and that in removed bits of leprous tissue in which autolysis has taken place, the Hansen rods continue to proliferate so long as there are amino-acids present, while in subsequent transplants from these bits of tissue, growth becomes more feeble as the protein split products decrease in amount. Further, the living chick embryo was thought a desirable medium since it is known that there is a large supply of split protein accessible to its growing cells. The question of susceptibility

[1] Holt, R. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 567.

[2] Duval, C. W., *J. Exp. Med.*, 1910, **12**, 649.

of the living chick embryo to leprosy and the adaptation of *B. leprae* to this environment was considered. In this connection, it is realized that very few lower animals are susceptible to *B. leprae* propagation.

The object of this study was to determine the time required for the growth and multiplication of *B. leprae* in the living chick embryonic tissue, the *modus operandi* of the invasion and the effects of colonization upon the living cells. That part of the study which deals with the time required for *in vivo* cultivation of *B. leprae* will be considered in this paper.

The method of Goodpasture[3] was utilized. In brief, fertile hens eggs were allowed to incubate in ordinary laboratory incubator at 38°-40°C. in the presence of moisture. The eggs were candled after incubation for 10 to 14 days. If development of the embryos had occurred, square windows were cut in the shells and shell membranes, taking care to avoid injury to all other membranes. Through these windows inoculations were made upon the chorio-allantoic membranes. Coverslips were then sealed over the holes in the shells with sterile paraffin-vaseline mixture. The reactions of the chorio-allantoic membranes and activities of the embryos were conveniently observed through the windows. Near the end of embryonic development, the embryos, membranes and egg substances were fixed *in situ* with Zenker's fluid or formalin, sectioned and stained with hematoxylin-eosin and by the Ziehl-Neelsen method for the demonstration of acid-fast bacilli.

The materials used for inoculations were emulsions of human leprous nodules in sterile Ringer's solution. The leprous tissue was received from Dr. O. E. Denney, Director, National Leprosorium, Carville, Louisiana. Some of these tissues were contaminated, while others were not. If contaminated, the emulsions were treated for one hour with 3% sodium hydroxide and then washed 3 times in Ringer's solution.

Macroscopic observations through the windows during the development of the embryos repeatedly revealed very definite inflammatory responses in the allantoic membranes as evidenced by a marked distension of the vessels and its distinct thickness and opaqueness.

Microscopic examinations of sections of the embryos, membranes and egg substances that were stained by Ziehl-Neelsen method gave no evidence of the multiplication of *B. leprae*. The acid-fast bacilli and "globi" observed were considered to be those placed there in the original inoculations since, by comparison with

[3] Goodpasture, E. W., *So. Med. J.*, 1933, **26**, 418.

the index inoculum-slides, there was no increase in number, no loosening of the "globi" and no change in morphology. Furthermore, the host reaction as noted in the hematoxylin-eosin stained sections was that of a sub-acute and chronic inflammation such as occurs about an inert foreign body. The tissue reaction was characterized by lymphoid, plasma and fibroblastic cells. Neutrophiles were conspicuous by their absence. Endothelial cells were present in and around the tissue particles of the inoculum. However, there was no evidence that these cells had phagocytized the *B. leprae* or that the latter had invaded them.

These results indicate to us that the multiplication of *B. leprae* does not occur *in vivo* in chick embryos within 7 to 11 days. Experiments are in progress in which successive transplants from one chick embryo to another are made. We hope to determine in this way if a longer period of incubation will result in *B. leprae* multiplication, host-cell invasion and intracellular colonization. In conclusion, we feel that living chick embryo may be satisfactory for the study of the *in vivo* behavior of *B. leprae*.

7259 C

Morphological Resemblance of the Rod Shaped Pigment of the Chick Retina to B. Leprae.

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During recent attempts to cultivate *B. leprae* in embryonic chick tissues,[1] an interesting observation was made on the close morphological resemblance of this microorganism in freshly excised human leprous tissue to the rod shaped pigment of the chick retina. This observation is considered worthy of note because of the possibility of confusing these bacilli and pigment rods when employing embryonic tissue as a culture medium. Furthermore, as Rivers[2] points out, the presence of the pigment rods may lead to confusion when smears are made to determine the sterility of the cultures. For this reason, he suggests removing the eyes of the embryo before making

[1] Holt, R. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 567, 643.

[2] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.