Pacific Coast Section

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8307 C

Effect of Heat on Viability of Mycobacteria leprae muris.

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Clegg[1] developed a method of cultivating *M. leprae,* using media on which amebae were growing, later heating the cultures to 60°C. for 30 minutes. This procedure destroyed the amebae and symbiotic bacteria and resulted in what Clegg believed to be a pure culture of *M. leprae.* Other workers have stated that on exposing *in vitro* the bacilli of leprosy to 60° for 10 minutes the bacilli are killed.[2] It has been suggested,[3] presumably on the basis of the latter observations, that human leprosy might be susceptible to fever therapy. Denney[4] is attempting to determine if pyrotherapy is of any value in the disease and, if so, whether the effect is a direct thermal one. At his suggestion the following experiment was undertaken.

A leproma was removed from a rat, under sterile conditions, ground with sterile sand and suspended in physiologic (0.9%) saline solution. Seven equivalent amounts were placed in sterile test tubes and exposed to the following temperatures for five hours: 17°, 37°, 39°, 41°, 43°, 50° and 60°C. To serve as test subjects for each respective temperature, 7 groups of 10 rats each were selected of approximately the same weight (average of 200 gm. each) and age, except the first control group (17°C.) which were younger animals, averaging 80 gm. each. One-half cc. of the respective heat-treated saline-suspended *M. leprae muris* was injected subcu-

1 Clegg, M. T., *Phil. J. Sci.,* 1909, **4**, 77, 403.

2 Alexandrescu, Marchoux, and Mezinescu. Quoted by Koch, F., *Zentr. f. Haut.-u. Geschlechts-krankh.,* 1932, **40**, 433.

3 Heiser, Victor, personal communication.

4 Denney, O. E., personal communication.

taneously into the right lower quadrant of the abdominal wall of each animal.

During the 5 months since the infected material was inoculated the following observations were made: Mycobacteria exposed to 17°C. and 37°C. produced palpable lesions at the injection-site in all animals within 3 months; organisms heated to 39°C. caused lepromata in 7 of 8 surviving rats in 3 months; 60% of the animals given material kept at 41°C. had lesions at the end of 3 months and 75% of the survivors within 4 months. Six of 7 surviving rats injected with the leprous suspension exposed to 43° had palpable leprous masses at the end of 4 months. Temperatures of 50° and 60°C. for 5 hours are sufficient, apparently, to attenuate the *Mycobacteria* and prevent the appearance of lesions, since no animal receiving this material showed signs of leprosy during the observation period of 5 months.

Weight gains were noted only in groups not developing lepromata, *i. e.,* 50°C. (average gain, 27 gm.) and 60°C. (average gain 180 gm.). In the controls given material kept at room temperature, however, an increase due to growth was noted. Intercurrent infection, usually pneumonia, accounted for the death of 26 of the 70 rats originally injected.

Summary. Temperatures in excess of 43°C. for 5 hours are required to kill *Mycobacterium leprae muris in vitro,* using rats as a test animal to determine viability over an observation period of 5 months. Fifty degrees C. for 5 hours seems sufficient to attenuate this *Mycobacterium.*

8308 P

Hemolytic Complement Albumin-Globulin Ratio.

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If fresh, cell-free, guinea pig serum in a test tube is repeatedly frozen and thawed without shaking or inverting the tube there will be seen, after a few freezings and thawings, a difference between the upper and lower portions of the tube. If the serum is tinged with hemoglobin the lower portion will be deep red and the upper portion colorless. If there is only a trace of hemoglobin an indica-