THE VIABILITY OF *MYCOBACTERIUM LEPRAE MURIUM* IN TISSUE STORED WITH DRY ICE

PHILIP C. EISMAN, SAM G. GEFTIC
AND R. L. MAYER

Ciba Pharmaceutical Products, Inc.
Summit, New Jersey

Although several workers (1, 5, 6) have reported success in the cultivation of *Mycobacterium leprae murium*, it is the general belief that the continued propagation of this organism can be achieved only by successive passages through susceptible animals (2, 4). Glover (3), in a careful study made with human and bovine tubercle bacilli, showed that in aqueous suspensions these organisms remained viable after storage at –76°C. for at least 180 days. Because these organisms, as well as a number of others including *Treponema pallida* and many viral and rickettsial agents, can be readily maintained in a viable state and without an appreciable alteration in virulence by storage in an environment of dry ice, we have investigated the suitability of this method for maintaining the Stefansky bacillus. There would be obvious advantages if one could use frozen stored material instead of being dependent upon freshly obtained lepromatous tissue. It would make available at any given time material capable of inducing an experimental infection in animals, thereby obviating the maintenance of a colony of "seed" animals in varying stages of infection.

On three occasions, lepromatous tissues developed subcutaneously in the shoulder areas of either rats or mice were aseptically excised and cut into a number of portions. Each piece was weighed in sterile pyrex test-tubes fitted with rubber stoppers and firmly sealed with adhesive tape. Within 30 minutes from the time of removal, the tissues were immersed in a "quick-freeze" bath of dry ice and acetone, then stored in a dry-ice chest. At varying intervals of time ranging from 1 to 42 weeks, samples of the frozen tissues were removed, thawed at 5°C., and prepared as a 10 per cent suspension in sterile distilled water. To eliminate bacterial contamination, 1,000 units of penicillin G were added for each milliliter of suspension, which was then kept at room temperature for two hours prior to injection into normal rats and mice.

Groups of 5 to 10 mice or 2 to 5 rats were inoculated with the suspension prepared from each of the stored lepromatous

tissues, 0.5 to 0.75 ml. being injected subcutaneously into each animal, in either the sternal area or the shoulder region. The mice were sacrificed at periods ranging from 4 to 22 weeks after inoculation, while the rat experiments were terminated after from 24 to 34 weeks.

In all instances the frozen lepromatous tissue proved capable of inducing lepromas of varying size at the site of inoculation, with no apparent loss in infectivity of tissue frozen for 42 weeks. Palpable lepromas generally appeared in mice in about 3 to 4 weeks, while in rats 9 or 10 weeks usually elapsed before definite lepromas could be felt.

RESÚMEN

Se demostró que material de leproma murino, sometido a congelación rápida en una mezcla de CO_2 sólido y acetona y mantenido a la temperatura del CO_2 sólido, mantuvo su infectividad por 42 semanas. Este método de mantener material para inoculaciones evitaría el tener que prover animales en varios estados de la infección para proveer material de inoculación.

REFERENCES

1. ASHBEL, R. and POLJAKOFF, A. *In vitro* cultivation of the rat leprosy organism. J. Infect. Dis. **83** (1948) 279-282.
2. CARPENTER, C. M. Murine leprosy; its usefulness as an experimental infection. Ann. New York Acad. Sci. **54** (1951) 101-106.
3. GLOVER, R. E. The effects of (*a*) freeze drying and (*b*) low temperature on the viability of *Mycobacterium tuberculosis*. J. Path. & Bact. **58** (1946) 111-114.
4. HANKS, J. H. Bacteriology of leprosy. Ann. New York Acad. Sci. **54** (1951) 12-19.
5. ROW, R. On the experimental transmission of rat leprosy in the mouse with the culture of *Myco. stefanski* isolated by symbiosis. Indian Phys. (Bombay) **8** (1949) 145 (cited from an abstract in Internat. J. Leprosy **18** (1950) 126).
6. SOUZA-ARAUJO, H. C. Four strains of cultures (two chromogenic and two nonchromogenic) of acid-fast bacilli isolated from rats and mice inoculated with emulsion of Stefansky's leproma from Institut Pasteur of Paris. Fifth Internat. Congr. Microbiol., Rio de Janeiro, Aug. 1950.