The Temperature Factor in the Growth of *M. Ulcerans*

[EDITOR'S NOTE: The importance of temperature in the growth of certain pathogenic mycobacteria has been stressed frequently. Many of them thrive best at temperatures below that of the human body, but a narrow range as respects variation in temperature appears to be of great significance. The following extract from a letter from Dr. Jean C. Tolhurst, Research Fellow in Bacteriology, Alfred Hospital, Prahan, Victoria, Australia, to Dr. Daniel H. Connor, Geographic Pathology Division, Armed Forces Institute of Pathology, Washington, D. C. is believed to be of sufficient interest in this respect to justify making its contents more widely available. Mr. Glen Buckle and Dr. Tolhurst are noted as the first to cultivate *Mycobacterium ulcerans* (MacCallum, P., Tolhurst, Jean C., Buckle, G. and Sissons, H. A. A new mycobacterial infection in man. *J. Path. Bact.* **60** (1948) 93-122). The correspondence partially reprinted below was with reference to a recently published article by Daniel H. Connor and H. Fletcher Lunn: Buruli ulceration. *Arch. Path.* **81** (1966) 183-189. It is presented here with the permission of Dr. Tolhurst and Dr. Connor.]

DEAR DR. CONNOR:

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Perhaps I should tell you a bit more about our incubators.

At times we were using all the available space in the incubators in our Department, naturally having been careful to check that the thermometers of the "37°" incubators did read 37°. We made attempts at cultivation at quite irregular intervals over many months, whenever we had a suitable rat to give us fresh peritoneal fluid. The culture tubes were incubated wherever there was space, as the convenience of our diagnostic work for hospital patients came first.

When a few tubes showed colonies of acid-fast bacilli we naturally hoped they were the organism we were looking for, but we could not prove it. We could not produce satisfastory subcultures and we could not repeat the event from new rat fluids. No one could claim to have grown

a new organism on such evidence. Months went by while we tried again. At that time the positive cultures were useless to us; later they were an impediment.

During a long period we had thought and talked about the possible significance of the temperature of the sites of the lesions in man and animals. Many scraps of evidence accumulated, but we were particularly impressed by the primary epididymal lesions in male rats, and the notion that the function of the scrotum is to keep the testes cool. In female rats we obtained no intra-abdominal lesions. The growth of the cultures mentioned above, at 37°C, seemed to refute these ideas.

Just then we had no rats at a suitable stage to obtain the fresh fluid which we assumed would be necessary for culture. Because we could not go on, it occurred to Glen Buckle to check the records and he saw that the growths were all associated with the one incubator. It was an anhydric type and he deduced that it might have an unsatisfactory heat circulation. He found that although its thermometer varied only insignificantly from 37°, the floor varied between 33° and 37° during each three or four hour heating-cooling cycle. In retrospect it appeared from the records that we had placed subcultures and "repeat" cultures in other more conveniently situated incubators. The evidence of the cultures was now in agreement with our ideas on temperature. As I said, we had no fresh fluid, but we had several fluids stored in the refrigerator; we attempted cultivation and this time we were successful.

It was the idea that the site of the lesions would be below 37°C that led us to examine the incubator and not vice versa. We thought this was an interesting series of ideas and observations.

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JEAN C. TOLHURST

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