REPRINTED ARTICLES

[EDITOR'S NOTE: The papers partially reprinted below, by F. W. Twort and G. L. Y. Ingram, represent a milestone in the laboratory culture of mycobacteria that were once considered difficult if not impossible to cultivate artificially. As such they are of unusual interest for all those interested in artificial culture of *Mycobacterium leprae*. It is worth noting that Twort had the leprosy bacillus in mind when he developed the idea that led to success in the case of *Mycobacterium johnei*. Indeed he believed, presumably erroneously, that he actually had cultivated the leprosy bacillus. Today the experiments of Twort and Ingram are basic in current efforts to provide *M. leprae* with accessory growth factors that might promote its multiplication on laboratory media otherwise unsuitable. Twort and Ingram did not attempt to explain the success of their procedure in more than general terms, which at the time, however, seemed logical and sufficient. The more recent experiments of Hanks and Morrison and their associates (*see* Hanks, J. H. The cultivation of *Mycobacterium leprae*. Search for a rational approach. *Internat. J. Leprosy* **33** (1965) 563-569, Part 2) have gone more deeply into the mechanism involved. Their investigations include studies of the essential ingredient, mycobactin, concerned in the growth of *M. johnei* and certain wood pigeon mycobacteria whose multiplication is limited by the same growth requirements. Still more recently Prof. Arych S. Olitski and associates in Jerusalem, Israel, (Maintenance of cytopathic activity of *Mycobacterium leprae* in Eagle's medium supplemented by mycobacterial extracts. *Israel J. Med. Sci.* **1** (1965) 1004-1005. *See also* note in *Internat. J. Leprosy* **33** (1965) 912) have reported some success in cultivation of the leprosy bacillus itself through appropriate use of this essential ingredient.]

A Method for Isolating and Growing the Lepra Bacillus of Man

Preliminary Note[1, 2]

F. W. Twort[3]

For a number of years different investigators have attempted to cultivate the lepra bacillus of man and the allied organisms found in the rat and other animals. It is not intended in this preliminary note to discuss the numerous papers which have been published from time to time from the various English, Continental and American laboratories. These papers deal with non-acid-fast bacilli, or with acid-fast bacilli growing quickly on ordinary media, which, in the opinion of the writer, are contaminating organisms, and not the true lepra bacillus. So far, no one has produced a culture of acid-fast bacilli isolated from a leper, and showing the characters of the lepra bacillus as found in the tissues of man. It was with the object of obtaining a pure living culture of the lepra bacillus that these investigations were undertaken.

The material used was the nasal discharge and scrapings from a typical leper.

[1] Reprinted from *Proceedings of the Royal Society of London, Series B*, **83** (1911) 156-158, with permission of the Royal Society.

[2] Communicated by Leonard Hill, F.R.S. Received September 30—read November 17, 1910.

[3] From the Laboratories of the Brown Institution, University of London.

The discharge showed large masses of lepra bacilli and a n u m b e r of contaminating micro-organisms. Firstly, most of the contaminations were killed by placing the discharge in a 2 percent solution of ericolin at 38° C. for one hour as recommended for the isolation of the tubercle bacillus[4]; then cultures were made from the sediment on to different media and incubated at 38°C.[5]

All the ordinary laboratory media, including Dorset's egg medium, gave negative results. A number of special media containing extracts of fresh gland and other tissues were tested next, the extracts being freed from any contaminating micro-organisms by passing them through a Doulton white filter; these also gave negative results.

In view of the close relationship between the tubercle bacillus and the lepra bacillus, it appeared highly probable that these two organisms would require the same chemical substances for building up their protoplasm, which could be elaborated from the ordinary media only by the tubercle bacillus. It was thought that if these substances could be supplied, already formed, to the lepra bacillus, it might grow, and the easiest method of supplying these substances would be by adding to some good medium the ground-up bodies of tubercle bacilli containing them. Accordingly, a number of tubercle cultures were taken and inoculated on to Dorset's egg medium; when sufficiently grown the tubes were steamed and the growth of tubercle scraped off the surface, care being taken to avoid the medium containing the waste products of the tubercle growth. The tubercle bacilli so obtained were ground up with glycerine and saline, steamed for half an hour and added to the yolk and white of new laid eggs in the following proportions:

Eggs, 75 parts; 8 percent sodium chloride, 25 parts; mix well and add tubercle bacilli, 1 percent; and glycerine, 5 percent, or less.

The medium was placed in test tubes, heated to 60° C. for one hour, and on the following morning incubated at 38° C. for six hours, and again heated in water bath at 60° C. for one hour, and set in slopes at 85° C.

The ericolinised nasal discharge of a leper was inoculated on to this medium, the inoculated tubes being capped with gutta-percha tissue and incubated at 38° C. After 24 hours the medium absorbed a quantity of the ericolin, so the material was lifted off with a platinum loop and rubbed over fresh tubes. The bacilli grew and were sub-cultured in pure growth, the bacilli growing in sub-cultures as fairly long thin beaded rods; the bacilli were well formed and quite acid-fast. The lepra bacillus inoculated on to this medium at first grows e x t r e m e l y slowly, but later growth becomes faster, marked microscopic evidence being obtained in about four weeks. To the naked eye, growth is only just visible after about six weeks, appearing as a colourless film along the needle track. Attempts to sub-culture on to ordinary laboratory media are always negative.

Experiments are now being carried out, using other organisms than the tubercle bacillus for making the medium, and also testing various micro-organisms on the medium.

In the near future it is hoped to prepare a vaccine from the ground-up lepra bacilli, for the treatment of man suffering from leprosy. An attempt to grow the rat lepra bacillus on the same medium will also be made if the material can be obtained.

In conclusion, I may note that, working with Mr. Ingram, I have also succeeded in isolating and growing the acid-fast bacillus found in the intestine of cows in Jöhne's disease. The first generation of this bacillus grows often long, with occasional branching and club formation; in subcultures it gradually grows smaller, and in the second or third generation is about the size

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[4] Twort, F. W. *Proc. Roy. Soc.* #B, **81** (1909) 248. [Ericolin is a resinous glucocidal substance obtained from *uva ursi* (bear berry), which was once used as a bactericidal substance destroying many microorganisms, but apparently not affecting mycobacteria.—EDITOR]

[5] Subsequent experiments have shown that 37° is a better temperature (November 19, 1910).

of the tubercle bacillus. The growth is only just visible to the naked eye, and subcultures on the ordinary laboratory media show no evidence of multiplication. Jöhne's bacillus grows somewhat more easily than Hansen's lepra bacillus; the bacilli being well formed and quite acid-fast. The cultures were incubated at 40° C. It is hoped, when sufficient material is obtained, to prepare a vaccine for diagnostic purposes.

Further details of these experiments will be published later.