samples are taken from animals for population analysis or for *in vitro* work.

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Multiplication of Hansen's Bacillus in Complex Symbiosis *in vitro*

The work reported here followed a chance observation made while attempting, without success, to grow *Mycobacterium leprae* in a culture of *Entamoeba terrapinae*.

A fragment of leproma (*L*.II), about 1 c.c. in size, had been incubated for eight days, at 25° C., in saline buffered at *p*H 7·0 with phosphates. It was thereafter inoculated into the following culture medium, called *M-E-R*: (*a*) 30 gm. of powdered skimmed milk were suspended in 200 ml. distilled water; this was autoclaved at 115° C. for 20 min. and cooled. (*b*) A whole egg (about 60 ml.) was aseptically homogenized either by hand-shaking in a flask containing glass beads or by the use of a mechanical mixer. (*c*) Milk (*a*) and egg (*b*) were mixed together and the mixture aseptically distributed (18 ml.) into sterile test tubes (dia. 25 mm.). (*d*) 2 ml. of an autoclave-sterilized solution of reductose (2·5 per cent in water) were aseptically added to each tube.

(A variant of medium *M-E-R*, used later on and called *M-E-R-M*, differed from it only in that solution (*a*) contained, in addition to the milk powder, 0·6 gm. gastric mucin.)

After incubation at 25° C. for several weeks, the microscopic examination of medium *M-E-R* inoculated with leproma *L*.II revealed the presence of an exceedingly large number of bundles of acid-fast bacilli recalling the typical 'globes' seen in the cells of lepromatous lesions.

However, only one out of three identical tubes inoculated likewise with fragments of the same leproma *L*.II gave such positive results. This suggested that contaminating microflora might be involved. From the single tube in which a multiplication of *M. leprae* had apparently occurred, four micro-organisms were isolated by standard procedures. Two were aerobes, an anthrax-like bacillus and a species of *Neisseria*. Two were anaerobes, an agar-liquefying organism and a gas-producing *Clostridium*.

My initial observation was repeated with three other specimens (*L*.I from the same patient as *L*.II, *L*.III and *L*.IV from two other patients) using the following procedure: (*a*) incubation of the fragment from leproma (about 1 c.c.) at 25° C. during at least 8 days in buffered saline (*p*H 7·0); (*b*) inoculation of the four micro-organisms together in a tube of medium *M-E-R* (or *M-E-R-M* in the case of specimen *L*.IV) and incubation during about 24 hr. (the medium is then clotted); (*c*) introduction of the macerated fragment (*a*) on the surface of (*b*) and incubation at 25° or 34° C., the tubes being tightly capped.

After one week, the clotted medium was liquefied and the proliferation of the symbionts could be stopped by the addition of either 'Sigmamycine' (a platinum loop of the dry product) or malachite green (1 ml. of a 0·5 per cent sterile solution in water) or both. However, this step was optional since it did not influence at all the evolution of *M. leprae* as observed monthly in Ziehl-stained smears.

In primary cultures, bundles of acid-fast bacilli increased and diminished in number alternately several times. They were preferably localized at the surface of the medium and in the ring which was progressively formed on the glass just over the surface. When occurring singly, bacilli were granular in appearance, but this happened very rarely as they were mostly seen in three-dimensional dense bundles or in one-plane clumps. Sometimes the agglomerates were rather loose and sometimes disintegrated into separate individuals in the immersion oil. There was no macroscopic difference between a culture in which *M. leprae* were multiplying and one inoculated with the symbionts only. Some observations, which, however, required further confirmation, led me to believe that the anthrax-like bacillus is the organism responsible for making medium *M-E-R* suitable for the growth of *M. leprae*.

Secondary cultures from the primary tubes were obtained in two ways. Either in medium *M-E-R*, using then a large inoculum representing at least one-fifth of the volume of culture, or on agar-media of various composition containing malachite green, using such an inoculum that, after partial desiccation, it left a heavy and creamy layer on the surface. Further sub-cultivation has so far been unsuccessful.

I now have on hand four suspensions very rich in bundles of *M. leprae* and aged respectively 12 (*L*.II), 11 (*L*.I and *L*.III) and 8 (*L*.IV) months. The possibility of subcultivating from them on an agar-medium gives us the hope of devising a method for the easier preparation and standardization of large amounts of lepromin.

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Rhamnose for Detection and Isolation of *Azotobacter vinelandii* Lipman

During investigations on the utilization of pentoses by *Azotobacter* spp., *A. vinelandii* proved to be the only species able to utilize rhamnose as sole carbon source. The experiments were carried out with a nitrogen-free nutrient solution containing mineral salts, 0·1 per cent agar and 1·0 per cent rhamnose as source of carbon and energy. The growth was estimated by comparison with check cultures without carbon source, and in a few cases the nitrogen fixation was determined by Kjeldahl analysis.

Twenty-eight strains of *A. chroococcum* and nine strains of *A. beijerinckii* were tested, and all were found completely unable to utilize rhamnose. Of *A. vinelandii* ten strains were tested, and nine grew as well or better with rhamnose as sole source of carbon as with glucose. One strain showed only slight, but unmistakable, growth with rhamnose as carbon source. In the nitrogen fixation experiments 14·1–15·8 mgm. nitrogen was fixed per gm. of