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Metabolism of the Tubercle Bacillus in Long's Synthetic Medium.*

THEODORE E. FRIEDEMANN AND FLORENCE B. SEIBERT.

From the Department of Medicine and the Otho S. A. Sprague Memorial Institute, University of Chicago, Chicago.

The rapid growth of mycobacteria in media which contain glycerol was first noted by Nocard and Roux.¹ The growth characteristics of the tubercle bacillus in such media have since been studied by many investigators. Synthetic media, similar to that of Long,² are now extensively used in studies of the metabolism of mycobacteria. Long and Finner³ demonstrated a direct relation between the initial concentration of glycerol and the maximum weight of bacilli which could be obtained in such a medium. Dingle and Weinzirl⁴ determined the nitrogen metabolism and made approximate analyses of the glycerol content. Similar determinations were made by Wedum.⁵ As yet no accurate measurement has been made of the consumption of glycerol and its relation to the mass of bacilli. Such data are given in the present communication.

Exactly 200 cc of sterile Long's synthetic medium,² with 6% of glycerol, were pipetted into sterile tall round bottles of 1 liter capacity. The bottles were loosely stoppered with cotton to facilitate exchange of gases. The bottles were placed on their side; they were inoculated and then incubated at 37.5°C.

At the end of each week one inoculated bottle and one uninoculated bottle were treated as follows: Exactly 50 cc of the clear fluid below the mass of bacilli at the surface were run into 5 cc of 1.0 *N* H₂SO₄.⁶ The bacilli were then filtered through a tared Gooch crucible, washed with cold water, and dried to constant weight at a low temperature. An excess of acid mercuric nitrate was added to a measured volume of the acidified sample, alkali was added until the reaction became neutral, and the volume was then brought to the mark. An aliquot of the filtrate was treated with CuSO₄ and

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¹ Nocard and Roux, *Ann. Inst. Past.*, 1887, **1**, 19.

² Long, E. R., and Seibert, F. B., *Am. Rev. Tuberc.*, 1926, **13**, 393.

³ Long, E. R., and Finner, L. L., *Am. Rev. Tuberc.*, 1927, **16**, 523.

⁴ Dingle, J. H., and Weinzirl, J., *J. Bact.*, 1932, **23**, 281.

⁵ Wedum, A. G., *J. Bact.*, 1936, **32**, 599.

⁶ Friedemann, T. E., *J. Bact.*, 1938, **35**, 527.

an excess of $\text{Ca}(\text{OH})_2$. The final filtrate was then oxidized with alkaline KMnO_4 , as described by Freeman and Friedemann,⁷ for the determination of glycerol.

It should be noted that all of the "blank" or uninoculated bottles and only one of the inoculated bottles, namely the one which was analyzed after one week of incubation, gave a precipitate immediately after addition of the mercury reagent. Such a precipitate is given by citric acid and by asparagine, both of which are constituents of the synthetic medium. This result indicates a rapid consumption of these constituents.

The results from an experiment with Strain 119 are given in Fig. 1. The dry weight of bacteria and the glycerol consumption increased rapidly after the first week of incubation. The glycerol was completely consumed at the end of about 3.5 weeks. The maxi-

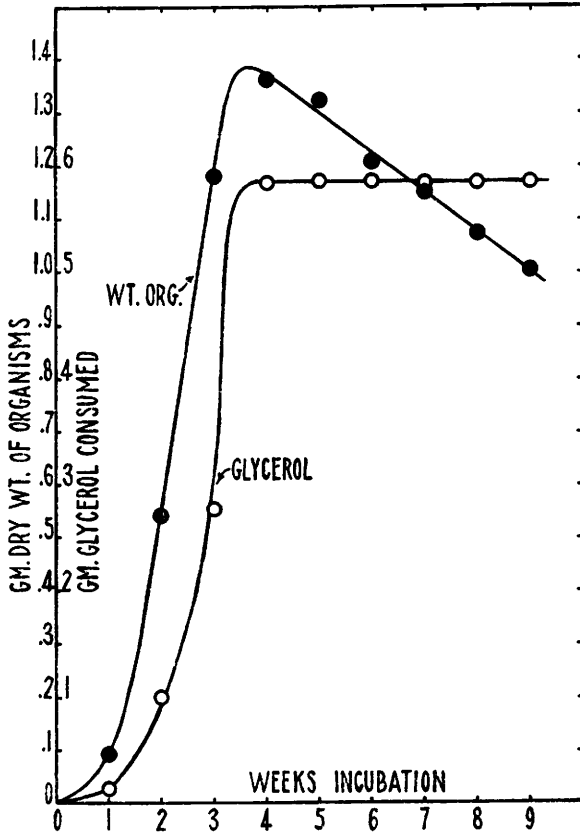


FIG. 1.

⁷ Freeman, S., and Friedemann, T. E., *J. Biol. Chem.*, 1935, **108**, 471.

imum weight of dry bacilli, 1.37 g per 100 cc of culture medium, was obtained at about this time. Thereafter the weight of organisms steadily diminished, due to rapid autolysis. The point at which autolysis was first noted corresponds with the time at which considerable quantities of tuberculin are usually found in the medium.

Quantitative determinations of lactic acid⁸ failed to reveal even traces in any of the cultures.

Conclusions. It is shown (1) that the rapid growth of the microorganisms is directly related to the consumption of glycerol and other constituents of the synthetic medium, and (2) that rapid autolysis occurs after exhaustion of the nutrients.

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Capillary Permeability in the Skin of the Rabbit.*

R. H. RIGDON. (Introduced by R. C. Avery.)

From the Department of Pathology, Vanderbilt University Medical School, Nashville, Tennessee.

The mechanism by which capillaries permit dyes and particulate matter to escape in increased amounts is not clearly understood. Many investigators have observed certain physiological and pathological changes in experimental animals in which the capillaries were more permeable than normal. Burrows¹ and Menkin² have stated that trypan blue and India ink when injected intravenously localize and concentrate in areas of inflammation. The latter has also shown that such substances when injected into areas of inflammation are retained longer than when they are injected into normal tissue.

Recent studies in this laboratory have shown that trypan blue and India ink do not always concentrate in areas of inflammation produced by the local application of xylol in the skin of rabbits. These substances concentrate in such areas only when they are injected into the circulation immediately or within a period of less than 5 hours following the application of the xylol. The fact that trypan blue and carbon particles fail to concentrate in xylol-treated areas

⁸ Friedemann, T. E., and Graeser, J. G., *J. Biol. Chem.*, 1933, **100**, 291.

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¹ Burrows, H., *Localization of Disease*, Wm. Wool and Co., 1932, New York.

² Menkin, V., *Physiol. Rev.*, 1938, **18**, 366.