AN EXPLANATION FOR THE ARITHMETIC LINEAR GROWTH OF MYCOBACTERIA [1]

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Arithmetic linear growth has been defined by Fisher *et al.* (1951) as a prolonged growth phase in which bacterial density increases as a straight line function of time. Fisher and Kirchheimer (1952) found this type of growth in 15 different strains of mycobacteria and proposed that arithmetic linear growth is a characteristic feature of members of the genus *Mycobacterium*. They suggested this phenomenon might be responsible for the relatively slow growth of mycobacteria and provide a basis for the elucidation of some physiologic properties of mycobacteria that distinguish them from nonacid fast microorganisms.

The concept of arithmetic linear growth would involve some pattern of reproduction and growth not readily explained in terms of binary fission. On the contrary, arithmetic linear growth of any organism would be expected if some essential nutrient were provided at a constant limiting rate. Data are presented in the present paper which show that cultures of mycobacteria and two other strict aerobes (*Micrococcus lysodeikticus* and *Sarcina lutea*) will grow logarithmically when they are aerated continuously by shaking and that arithmetic linear growth is obtained only when the cultures are not aerated.

MATERIALS AND METHODS

The following species of bacteria were used: The BCG strain of *Mycobacterium tuberculosis* var. *bovis*, *Mycobacterium phlei*, *Mycobacterium smegmatis*, *Micrococcus lysodeikticus*, and *Sarcina lutea*. All of the strains were typical with respect to morphology and growth characteristics. Strains of mycobacteria grew well on standard synthetic media and were thus typical with respect to nutrient requirements.

For experiments with species of *Mycobacterium* inocula from actively growing cultures were

grown in a modified Dubos medium which contained the following per liter: potassium monobasic phosphate, 1.0 g; sodium dibasic phosphate, 2.5 g; asparagine, 2.0 g; casein hydrolyzate, 1.0 g; ferric ammonium citrate, 0.05 g; magnesium sulfate, 0.005 g; "tween 80",[2] 0.4 ml; and glycerine, 10 ml per liter. The medium was adjusted to pH 6.8 and autoclaved in 100 ml quantities. Five per cent sterile bovine serum or 0.25 per cent sterile bovine albumin (fraction V)[3] was added to the cooled medium.[4] Nutrient broth (Difco) was used for experiments with *S. lutea* and *M. lysodeikticus*.

Growth experiments were carried out at 37 ± 1 C using either stationary cultures or cultures which were aerated continuously by means of a rotary shaker.[5]

Standard Klett tubes containing 7.0 ml of medium and covered with aluminum caps were employed for stationary cultures. Turbidities were determined using a Klett-Summerson colorimeter with a 660 mμ filter. Aliquots were removed aseptically from the aerated flasks for turbidity determinations. Tubes always were shaken before reading to disperse the organisms.

RESULTS

Arithmetic linear growth of *Mycobacterium tuberculosis*, strain BCG, as described by Fisher and Kirchheimer (1952) was obtained in stationary cultures. Similar growth curves were obtained with stationary cultures of *M. phlei* and *M. smegmatis*. This pattern of growth suggested that some essential factor might be available only at a constant limiting rate.

When dilute methylene blue was added to a

heavy stationary culture of BCG in a Klett tube, the bottom 90 per cent of the contents was decolorized within a period varying from several minutes to one hour. Thus, only approximately 10 per cent of the medium in these tubes would have an O/R potential favorable for optimum growth.

Typical arithmetic linear growth curves resulted when *M. lysodeikticus* was grown in a stationary tube culture (figure 1), whereas typical logarithmic growth curves were obtained when the same organism was grown on a rotary shaker.
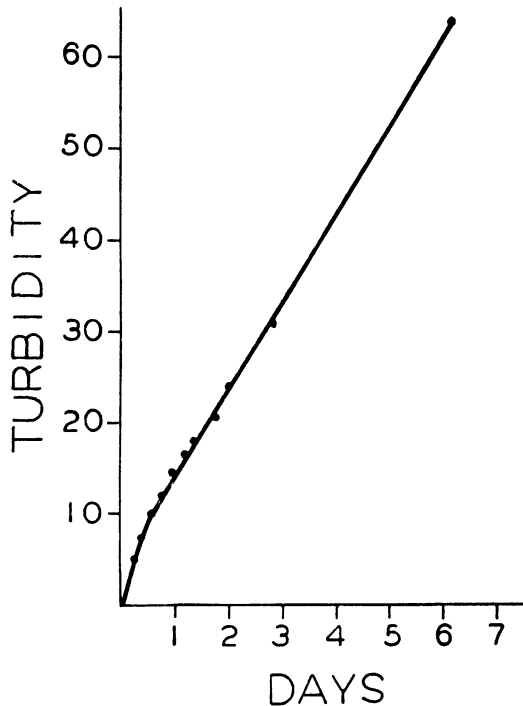

Figure 1. Arithmetic linear growth of *Micrococcus lysodeikticus* in stationary culture (nutrient broth).

Similar results were obtained with *S. lutea*. Typical logarithmic growth curves for each member of the genus *Mycobacterium* could be obtained when adequate amounts of oxygen were supplied (figure 2).

DISCUSSION

The concept of arithmetic linear growth proposed by Fisher and Kirchheimer (1952) for the genus *Mycobacterium* can be explained on the basis that limited access of oxygen to stationary cultures is the rate controlling factor. This ex-

planation is supported further by the observations of Novy and Soule (1925) who demonstrated that both growth and oxygen consumption of tubercle bacilli were limited by reduced oxygen tensions. In addition the work of Rahn and Richardson (1941) clearly demonstrates that the rate of oxygen diffusion in stationary cultures is not nearly sufficient to provide an entire culture of an aerobic organism with oxygen.
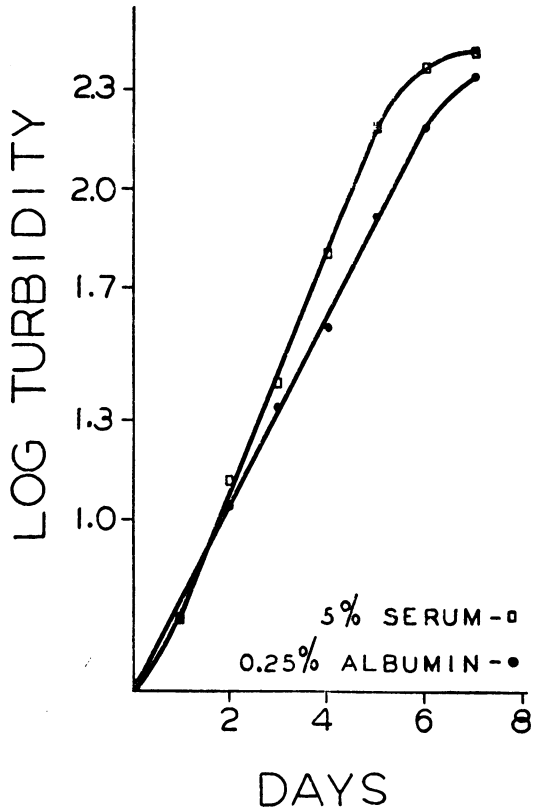

Figure 2. Logarithmic growth of the BCG strain of *Mycobacterium tuberculosis* var. *bovis* in an aerated culture (modified Dubos medium).

Fisher and Kirchheimer (1952) reported that studies in their laboratory failed to reveal an arithmetic linear growth curve for such nonacid fast organisms as *Micrococcus pyogenes* var. *aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Aerobacter aerogenes*, and *Escherichia coli*. These nonacid fast organisms, however, are facultative anaerobes (Breed *et al.*, 1948) and should grow logarithmically either in the presence or absence of oxygen.

SUMMARY

The evidence demonstrates that the rate of growth of obligate aerobes in stationary cultures is a function of the rate at which oxygen diffuses into the medium since the rate of oxygen consumption exceeds the rate of its diffusion. In contrast, typical logarithmic growth curves were obtained when the cultures of mycobacteria and *Micrococcus lysodeikticus* and *Sarcina lutea* were aerated adequately with a rotary shaker.

REFERENCES

BREED, ROBERT S., MURRAY, E. G. D., AND HITCHENS, A. PARKER 1948 *Bergey's manual of determinative bacteriology*. 6th edition. The Williams and Wilkins Company, Baltimore, Md.

FISHER, M. W., AND KIRCHHEIMER, W. F. 1952 Studies on the growth of mycobacteria. I. The occurrence of arithmetic linear growth. Am. Rev. Tuberc., **66**, 758–761.

FISHER, M. W., KIRCHHEIMER, W. F., AND HESS, A. R. 1951 The arithmetic linear growth of *Myobacterium tuberculosis* var. *hominis*. J. Bact., **62**, 319–322.

NOVY, F. G., AND SOULE, M. H. 1925 Microbic respiration. II. Respiration of the tubercle bacillus. J. Infectious Diseases, **36**, 168–232.

RAHN, OTTO, AND RICHARDSON, G. L. 1941 Oxygen demand and oxygen supply. J. Bact., **41**, 225–249.