AN EXPLANATION FOR THE ARITHMETIC LINEAR GROWTH OF MYCOBACTERIA

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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 lysodeikiticus 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 lysodeikiticus, 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", 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) was added to the cooled medium. Nutrient broth (Difco) was used for experiments with S. lutea and M. lysodeikiticus.

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.

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 m$\mu$ 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

1 Proprietary name of polyoxyethylene derivative of sorbitan monoooleate, Atlas Powder Co., Wilmington, Delaware.


3 Bovine serum and bovine albumin were sterilized by Seitz filtration.

4 Clay-Adams Co., Yankee Rotator, Cat. no. A-2275.
heavy stationary culture of BCG in a Klett tube, the bottom 90 per cent of the contents was de-colorized 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.

![Graph of Arithmetic Linear Growth](image1)

*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 explanation 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.

![Graph of Logarithmic Growth](image2)

*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. *au-reus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Aerobacter aerogenes*, and *Escherichia coli*. These non-acid 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


