STUDIES ON THE METABOLISM OF MYCOBACTERIUM TUBERCULOSIS

IV. The Effect of Fatty Acids on the Growth of M. tuberculosis var. hominis[1]

ANNE S. YOUMANS and GUY P. YOUMANS

Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois

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In previous studies fatty acids have been found either to inhibit growth or to be inadequate as carbon sources for the growth of tubercle bacilli in synthetic or semisynthetic media (Seiji, 1935; Cutinelli, 1941; Dubos, 1947; Saz, 1949). Dubos (1947), however, found that the addition of 0.1 to 0.5 per cent crystalline serum albumin to media containing single long chain fatty acids or esters would decrease or remove the toxicity of the fatty acids, thereby permitting growth of the organisms.

In contrast to the findings obtained from growth studies, certain fatty acids have been found to stimulate the respiration of mycobacteria (Loebel *et al.*, 1933; Cutinelli, 1940; Edson and Hunter, 1947; Saz, 1949; Lindsay *et al.*, 1950; Oginsky *et al.*, 1950; Geronimus and Birkeland, 1951). Bernheim (1941) observed that the oxygen uptake was increased more by the sodium salts of the lower molecular weight fatty acids than by the sodium salts of the higher molecular weight fatty acids, and that the amount of oxygen uptake was proportional to the concentration of the acid. Ozeki (1944), however, found that the lower fatty acids (1 through 8 carbons) markedly inhibited the respiration of tubercle bacilli unless low concentrations were employed. Bergström *et al.* (1946) observed that with the unsaturated fatty acids there was a certain limiting concentration above which the oxygen consumption of a human strain of mycobacteria was practically abolished, and below which the respiration of the organisms was not affected.

The present study is concerned with a reinvestigation of the effect of certain fatty acids on the growth of the virulent strain, H37Rv, of *Mycobacterium tuberculosis* var. *hominis* when employed in a wide range of concentrations and as single carbon sources in a basal synthetic medium.

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METHODS

The rate of growth of the H37Rv strain of *M. tuberculosis* var. *hominis* was measured quantitatively by the small inocula technique described previously (Youmans and Youmans, 1949).

The basal synthetic medium consisted of a modified Proskauer and Beck synthetic medium (Youmans, 1946) from which the usual carbon source, glycerol, was omitted. The lower molecular weight fatty acids (through capric acid), which are relatively soluble in water, were incorporated into the basal medium in the highest concentration employed, and fourfold dilutions then were made in the basal medium. The remaining higher molecular weight fatty acids were dissolved first in 1.0 or 2.0 ml of 95 per cent ethyl alcohol, and 100 ml of basal medium, previously heated to boiling, were added to this solution. The pH was adjusted to 7.0 with 40 per cent NaOH and dilutions then made rapidly using medium heated to boiling. A control series of the same dilutions of alcohol in the basal medium and in the basal medium plus glycerol was included. The various dilutions of each fatty acid and the alcohol immediately were tubed in 5 ml amounts into 18 by 150 mm pyrex test tubes which had been rinsed previously with double glass-distilled water. The tubed media were sterilized by autoclaving at 15 lb for 20 minutes. The inoculation of the media with each of the inocula (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} mg) of the H37Rv strain, the examination of the cultures thereafter, and the calculation of the growth rates followed the procedures described in detail in the first paper of this series (Youmans and Youmans, 1953).

RESULTS

The results of the growth studies are listed in table 1. When growth of the H37Rv strain occurred from all five inocula, or from all inocula except the smallest (10^{-6} mg), the results are

TABLE 1

Growth of Mycobacterium tuberculosis var. hominis, strain H37Rv, in a basal synthetic medium containing fatty acids

COMPOUND	GENERATION TIME IN HOURS									
	Concentration of compound in per cent									
	1.0	0.25	0.06	0.016	0.004	0.001	0.00025	0.00006	0.000015	0.000004
Formic acid	0	0	0	0	0	0	0	0		
Acetic acid	0	10^{-2}*	25.2 hr	25.2 hr	0					
Propionic acid	0	0	27.6 hr	10^{-3}*	0	0	0	0		
Butyric acid		0–10^{-3}*	24.0 hr	10^{-3}*	10^{-2}*	0	0	0		
Caproic acid		0	0	24.0 hr	10^{-3}*	10^{-2}*	0	0		
Caprylic acid			0	0	0	22.7 hr	10^{-3}*	10^{-2}*	0	
Capric acid			0	0	0	0	22.7 hr	10^{-2}*	10^{-2}*	0
Lauric acid				0	0	0	10^{-2}*	10^{-2}*	10^{-2}*	0
Myristic acid					0	0	0	10^{-2}*	0	0
Palmitic acid					0	0	0–10^{-2}*	0–10^{-2}*	0	0
Stearic acid					0	0	10^{-2}*	10^{-2}*	0	0
Oleic acid					0	0	+	+	+	0
Linoleic acid					0	0	10^{-2}*	10^{-2}*	10^{-2}*	0
Linolenic acid					0	0	0	10^{-2}*	0	0

Controls: Basal medium alone—no growth; basal medium + glycerol 2.0%—generation time 21.7 hr; basal medium + dilutions of ethyl alcohol ranging from 2.0% through 0.000005%—no growth; basal medium + glycerol 2.0% + dilutions of ethyl alcohol ranging from 2.0% through 0.000005%—generation time 21.7 hr.

* Smallest inoculum (in milligrams) which grew; 0—no growth; +—results irregular (see text).

expressed in terms of the actual generation time in hours. However, with most of the concentrations of the fatty acids employed, growth, when it did occur, was limited to those tubes containing one or both of the two largest inocula. Since these are insufficient data to permit accurate estimation of the growth rate, in these cases only the smallest inoculum which grew is given in the table.

The tests were repeated several times with each fatty acid, and no difficulty was encountered obtaining reproducible results except with oleic acid. The tests with oleic acid were performed six separate times with the following results: With an oleic acid concentration of 0.00025 per cent, growth of all inocula occurred in only one instance and the generation time was 24.0 hours. There was no growth of any inoculum at this oleic acid concentration in the remaining 5 tests. At a concentration of 0.00006 per cent, growth with a generation time of 24.0 hours occurred three times while no growth was observed in 3 trials. At a concentration of 0.000015 per cent, either no growth at all was observed or only growth of the 10^{-2} mg inoculum.

Under the conditions of the experiments, growth occurred in certain sharply limited concentrations of some of the fatty acids. Of these, caprylic and capric acids were the only ones which permitted growth at a rate comparable to that obtained with glycerol as the carbon source. Certain concentrations of acetic, propionic, butyric and caproic acids each also served as carbon sources for all or nearly all inocula. Growth, however, was not as rapid as with caprylic and capric acids. In the presence of the remaining fatty acids, only the largest inoculum (10^{-2} mg) was able to grow with the exception of formic acid where no growth occurred at all.

DISCUSSION

These results show that certain fatty acids, in low concentrations, will serve as carbon sources for the growth of virulent human type tubercle bacilli (strain H37Rv) even in the absence of a protective substance such as albumin.

The failure of previous investigators (Seiji, 1935; Cutinelli, 1941; Dubos, 1947; Saz, 1949) to obtain similar findings probably resulted from the use of too high a concentration of fatty acid or from the inoculation of too small a number of organisms, or both. There is for each fatty acid only a narrow range of concentrations in which growth of small inocula will occur.

The fatty acids of lower molecular weight stimulated the growth of mycobacteria more than the fatty acids of high molecular weight. Likewise Bernheim (1941), Cutinelli (1941), and Edson and Hunter (1947) found that the respiration of mycobacteria was stimulated more by the lower fatty acids than by the higher fatty acids. Cutinelli (1940) observed that the fatty acids from C_7 to C_{11} were oxidized most rapidly. In the present study, the fatty acids, C_8 and C_{10} (caprylic and capric acids), stimulated the growth of the organisms more than any of the other fatty acids examined. Formic acid did not support growth, nor did it stimulate the oxygen uptake of the H37 strain, the bovine B_1 strain (Bernheim, 1941), or *M. ranae* (Edson and Hunter, 1947).

Of interest is the finding that when growth of all inocula occurred, it was generally in only one of the fatty acid concentrations employed. Moreover, up to the C_{12} fatty acid (lauric) there was an inverse relationship between the molecular weight of the fatty acid and the concentration which allowed the fastest growth. The well known direct relationship between the molecular weight of fatty acids and their bacteriostatic activity also is evident from the tabulated data.

SUMMARY

It was found by employing a wide range of concentrations of a number of fatty acids in a simple chemically defined medium that, with the exception of formic acid, the fatty acids employed would support the growth of virulent human type tubercle bacilli by varying degrees. There was, however, only a narrow range of concentrations of each fatty acid which would support growth. The lower fatty acids supported the growth of the organisms more readily than the higher fatty acids. Caprylic and capric acids stimulated the rate of growth to a degree similar to that found in the presence of 2.0 per cent glycerol.

Among those fatty acids having 12 carbon atoms or less, an inverse relationship appears to exist between the molecular weight and the concentration of fatty acid which would stimulate growth.

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