STUDIES ON THE METABOLISM OF MYCOBACTERIUM TUBERCULOSIS

I. The Effect of Carbohydrates and Alcohols on the Growth of Mycobacterium tuberculosis var. hominis[1]

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Received for publication June 9, 1952

Although it has been seventy years since Koch first isolated *Mycobacterium tuberculosis* var. *hominis*, our knowledge of the suitability of various carbon sources for the growth of this organism is far from complete. There have been numerous publications on the effect of carbohydrates and alcohols on the growth and respiration of mycobacteria, but relatively few of these have been conducted with virulent strains of *M. tuberculosis* var. *hominis*.

Using a synthetic basal medium, Proskauer and Beck (1894) examined the effect of a number of carbon sources on the growth of tubercle bacilli. They observed that there was no growth when 1.0 per cent concentrations of mannose, sucrose, lactose, maltose, and raffinose were employed as the only carbon sources. Nor, under similar conditions, did the alcohols, *iso*-dulcitol, mannitol, and dulcitol, in concentrations of 0.6 per cent permit growth. Slight growth did occur in the presence of a 1.0 per cent concentration of levulose, and growth was questionable in the presence of the same concentration of glucose. However, on the addition of 1.5 per cent glycerol to the basal medium, the tubercle bacilli grew well in the presence of each of the above carbohydrates and alcohols. Gamble and Herrick (1922) found that the utilization of glucose by five strains of tubercle bacilli, which included the H37 and one other human type strain, was roughly parallel to the amount of growth obtained. A year later, Frouin and Guillaumie (1923) employed a synthetic medium containing 1.0 per cent glycerol and reported that three strains of tubercle bacilli, a human, a bovine, and an avian type, utilized lactose, maltose, saccharose, and trehalose when each was present in a concentration of 0.5 per cent. Kondo (1925) found that both glycerol and glucose supported the growth of a human type strain of tubercle bacillus, but that fructose, arabinose, and mannitol did not. He felt that the tubercle bacilli could utilize only a few of such substances. Weinzirl and Knapton (1927) employed fifteen strains of mycobacteria, including seven strains of *M. tuberculosis* var. *hominis*, and observed that upon the separate addition to Long's synthetic medium of glucose, lactose, and mannitol in a concentration of 1.0 per cent and of glycerol in a concentration of 5.0 per cent, only the medium containing glycerol turned acid. They therefore felt that only glycerol was utilized.

Merrill (1930, 1931) reported upon the carbohydrate metabolism of fourteen strains of the genus *Mycobacterium*, including the human type strains H37 and M-1. In the work reported in 1930, a beef infusion broth was employed as the basal medium to which each of the sugars was added. At the end of an unspecified period of incubation, quantitative determinations of the amount of sugar remaining in the cultures were done by Shaffer-Hartman blood-sugar method. He found that the strain H37 utilized glucose, fructose, arabinose, and galactose but did not utilize sucrose, lactose, and maltose. In his 1931 publication, Merrill stated that in a synthetic basal medium in which the compound being examined was the sole source of carbon, the strain H37, after 75 days of incubation, grew only in the presence of glucose and glycerol. No growth was observed in the presence of frustose, galactose, arabinose, maltose, lactose, sucrose, raffinose, inulin, mannitol, ethyl alcohol, salicin, sodium lactate, sodium citrate, and sodium acetate. The concentration employed was in each case 0.5 per cent. The M-1 strain grew only in the presence of glycerol and sodium acetate.

Model (1933) by chemical analysis found that human type tubercle bacilli utilized levulose when grown in a synthetic medium containing glycerol,

[1] This investigation was aided, in part, by a research grant from Parke, Davis & Company, Detroit 32, Michigan.

but levulose did not support growth in the same medium containing no glycerol.

Wedum (1936), using quantitative analysis for reducing sugar, found that human, bovine, and BCG types of *M. tuberculosis* utilized glucose, arabinose, xylose, and maltose, but not lactose. In these studies the organisms were grown in both sterile beef heart infusion broth and in Long's medium containing 0.5 and 2.5 per cent glycerol, respectively. To these media were added sterile solutions of the carbohydrates to make final concentrations of 0.45 per cent for the monosaccharides and 0.70 per cent for the disaccharides. The cultures were incubated at 37 C for 4 months.

Respiratory studies to determine the effect of carbohydrate on *M. tuberculosis* var. *hominis* have been made by Loebel, Shorr, and Richardson (1933) and by Nakamura (1938). Loebel and coworkers observed a limited amount of growth and an increase in the respiration of the H37 strain after a period of starvation when 5.0 per cent glucose was substituted for glycerol in Long's medium. This was not as great as that obtained in the presence of glycerol. They noted no effect on respiration by concentrations of glucose below 0.1 per cent. The presence of levulose, mannose, inositol, and arabinose in concentrations of 5.0 per cent produced no significant increase in the respiration of this strain. Nakamura (1938) observed that none of the following sugars and alcohols when added separately in several concentrations to a phosphate buffer had any effect on the respiration of a human strain of *M. tuberculosis*: glucose, galactose, levulose, mannose, maltose, raffinose, starch, glycogen, inulin, ethyl alcohol, erythritol, or mannitol. However, glycerol, especially in concentrations of 5.0 and 10.0 per cent, stimulated respiration of this strain.

Although many of the previously reported results appear to be in conflict, certain findings in common can be recognized also. First, all investigators agree that glycerol is utilized and supports rapid growth of mycobacteria. Second, the majority found that glucose is also a suitable carbon source although apparently not as good as glycerol. Third, *M. tuberculosis* var. *hominis*does utilize a variety of carbohydrates when grown in a medium which also contains glycerol but apparently does not grow in the presence of these utilizable sugars when glycerol is omitted from the medium.

In the work reported by previous investigators, techniques were not employed which permitted quantitative evaluation of the rate of growth. Furthermore, in many cases only a small number of carbohydrates or alcohols was employed and then frequently in only one concentration. In addition, the strains of *M. tuberculosis* var. *hominis* used frequently were not well defined as to virulence and variety.

In order to obtain definitive information, it seemed advisable to conduct growth studies employing a large number of carbohydrates and alcohols in a wide range of concentrations, a strain of *M. tuberculosis* var. *hominis* of high standard virulence, and a technique which would permit estimation of the actual growth rate.

METHODS

The effect of 24 carbohydrates and polyhydric alcohols on the growth of *M. tuberculosis* var. *hominis* (strain H37Rv) was tested utilizing the small inocula method previously described (Youmans and Youmans, 1949).

The basal culture medium employed was the modified Proskauer and Beck (Youmans, 1946) from which the normal carbon source, glycerol, was omitted. The compounds to be tested were incorporated in this medium in the highest concentration desired, sterilized by filtration through sintered glass filters, and lower concentrations prepared aseptically in sterile basal medium. Each concentration of each compound was tubed then aseptically in 5.0 ml amounts into sterile 20 by 150 mm pyrex test tubes. Sets of 3 tubes of each concentration of each compound were inoculated, respectively, with 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} mg of a mechanically prepared suspension of a young, actively growing, surface pellicle culture of the highly virulent H37Rv strain of *M. tuberculosis* var. *hominis*. Following inoculation, the cultures were incubated at 37 C for 5 weeks and examined daily for the appearance of visible growth. From the data so obtained, the rate of growth could be estimated (Youmans and Youmans, 1949). A minimum of two such experiments was conducted with each carbohydrate or alcohol employed.

RESULTS

In table 1 are listed the carbohydrates and polyhydric alcohols tested and the concentration of each employed. Also given is the generation time in hours of *M. tuberculosis* var. *hominis*

TABLE 1

The effect of various carbohydrates and polyhydric alcohols on the rate of growth of Mycobacterium tuberculosis var. hominis

COMPOUND	GENERATION TIME IN HOURS						
	Concentration of compound in per cent						
	4.0	2.0	1.0	0.25	0.06	0.016	0.0
I. Monosaccharides							
A. Pentoses							
D(−)Arabinose	0		0	0	0	0	
L(+)Arabinose	0		0	0	0	0	
D(+)Xylose	0		0	0	0	0	
D-Ribose	0		0	0	0	0	
D(−)Lyxose	0		0	0	0	0	
L(+)Rhamnose	0		0	0	0	0	
B. Hexoses							
D(−)Glucose	10^{-2}*	24.0	24.0	10^{-2}*	0	0	
D(+)Mannose	0		0	0	0	0	
D(−)Galactose	0		0	0	0	0	
Fructose	0		0	0	0	0	
II. Disaccharides							
D(+)Maltose	27.6†		27.6†	27.6†	0	0	
Lactose	0		0	0	0	0	
Sucrose	0		0	0	0	0	
III. Trisaccharides							
D(+)Raffinose	0		0	0	0	0	
IV. Polysaccharides							
Inulin	ppt		ppt	ppt	0	0	
V. Polyhydric alcohols							
Glycerol	21.7	21.7	21.7	21.7	24.0†	24.0†	
Trimethylene glycol	0		0	0	0	0	
Propylene glycol				0	0	0	
Triethylene glycol			0	0	0	0	
L-Arabitol			0	0	0	0	
Sorbitol			0	0	0	0	
Mannitol			0	0	0	0	
Dulcitol			0	0	0	0	
VI. None							0

ppt = precipitate
0 = no growth
* = smallest inoculum (in milligrams) which grew
† = slight growth

(strain H37Rv) in those cases where growth occurred.

Of the 24 carbohydrates and polyhydric alcohols used, evidence of growth was obtained in the presence of only three: glycerol, glucose, and maltose.

DISCUSSION

The results demonstrate conclusively that *M. tuberculosis* var. *hominis* (strain H37Rv), regardless of the concentration of substrate or the size of the bacterial inoculum, was unable to utilize for growth any of the 24 carbohydrates or alco-

hols except glycerol, glucose, and possibly maltose. These results confirm the more limited and less quantitative growth studies of Merrill (1931), Kondo (1925), Model (1933), and Proskauer and Beck (1894).

In contrast, it has been demonstrated previously that when growing in a medium containing glycerol, human type tubercle bacilli are able to utilize lactose, maltose, sucrose, and trehalose (Frouin and Guillaumie, 1923); levulose, galactose, and arabinose (Merrill, 1930); levulose (Model, 1933); arabinose, maltose, and xylose (Wedum, 1936).

Apparently *M. tuberculosis* var. *hominis* when growing in a medium containing a readily available source of energy, such as glycerol, is able to metabolize certain carbohydrates which cannot be utilized when the carbohydrate in question is the sole available carbon source. The mechanism of this phenomenon is not clear but would appear to be worthy of further investigation.

The results of our study indicate that glucose was not as suitable a carbon source as glycerol. In similar concentrations, the rate of growth was slower than with glycerol and, furthermore, glucose was not utilizable in as wide a range of concentrations as was glycerol. These results give quantitative verification to the reports of Proskauer and Beck (1894) and Merrill (1931) which indicated that glucose was a less suitable carbon source.

Although growth was consistently obtained in the higher concentrations of maltose employed, the significance of the results with this carbohydrate should be viewed with some reservation. It is possible that glucose, present as an impurity, may have been responsible for the growth which occurred in the medium containing this carbohydrate.

SUMMARY

The H37Rv strain of *Mycobacterium tuberculosis* was able to utilize for growth only glycerol, glucose, and possibly maltose, out of the 24 carbohydrates and polyhydric alcohols employed as sources of carbon.

Growth in the medium containing glycerol was more rapid than in the same medium containing glucose or maltose, and more rapid in the medium containing glucose than in the medium containing maltose.

Glycerol also supported growth in a wider range of concentrations than either glucose or maltose.

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