STUDIES ON CARBON METABOLISM OF ORGANISMS OF THE GENUS MYCOBACTERIUM

III. END PRODUCTS OF CARBOHYDRATE UTILIZATION AS DETERMINED IN SYNTHETIC MEDIA CULTURES

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Fermentation of carbohydrates by the majority of bacteria is accompanied by the accumulation of acid cleavage products in the medium. The reaction of the medium in such cases thus changes towards increased acidity. It was pointed out in a previous report (1930) that such an accumulation of acid cleavage products in carbohydrate broth cultures of the Mycobacteria does not occur when carbohydrates are utilized. The cultures in all cases become progressively more alkaline regardless of the degree of carbohydrate utilization. A mechanism of utilization differing somewhat from the usual type, which is characterized by incomplete cleavage of the carbohydrate, was thus indicated. From the results obtained it appeared that whenever the Mycobacteria attack a carbohydrate molecule a complete cleavage results, the end products of such cleavage being carbon dioxide and water with possibly some of the carbon going into the construction of the bacterial cells.

When a study was made of reaction changes induced by growth of Mycobacteria in a synthetic medium it was found that when carbohydrates constituted the carbon source the reaction changed towards increased acidity (1931). The question thus arose whether or not acid cleavage products were formed from the carbohydrates under these methods of experimentation. The following experiments were performed in an effort to determine the fate of the carbon which was added in the form of a carbohydrate to a synthetic medium.

METHODS

A medium containing the following ingredients dissolved in distilled water was used for these determinations:

	per cent
$NaCl$	0.5
$(NH_4)_2SO_4$	0.5
$MgSO_4$	0.005
K_2HPO_4	0.4
Glucose	1.0

The various ingredients were weighed out into a volumetric flask which was then filled to the mark with distilled water. Immediately after solution of all the reagents had been effected the medium was sterilized by filtration and subsequently pipetted accurately, 10 cc. per tube. Incubation at 37°C. for one week followed in order to rule out the presence of contaminants.

It will be noted that potassium phosphate is added to twice the concentration used in the work previously reported (1931). This increases the buffer effect and thereby prevents too rapid an increase in acidity with its associated inhibition of growth.

The species of Mycobacterium studied are listed in the table of results. In addition several non-acid fast organisms were studied such as *B. coli*, *Staphylococcus aureus*, *B. typhosus*, *B. subtilis*, and *B. proteus*.

After inoculation the tubes were placed in 1 liter Florence flasks to which had just previously been added an accurately measured amount of 0.2 N barium hydroxide. The flask was stoppered tightly employing specially prepared rubber stoppers as used in the former work (1930). Uninoculated tubes of media in flasks containing the same amount of barium hydroxide were used as controls.

At the termination of incubation the following determinations were made:

1. The carbon dioxide liberated from the culture was determined by titration of the residual barium hydroxide employing thymolphthalein as indicator. The difference multiplied by the factor 4.4 gave the milligrams of carbon dioxide liberated. Since all the cultures were acid in reaction no carbon dioxide was

retained in the medium. Repeated determinations demonstrated that wherever the pH was below 7.0 there could be no carbon dioxide demonstrated in the culture medium.

2. The titratable acidity of the culture medium was next determined. One-tenth cubic centimeter of 0.1 per cent phenol red was added to each tube. In all cases the medium was definitely acid. Then, employing N/40 sodium hydroxide, the reaction of the culture was brought to that of the uninoculated control.

3. The medium in each tube was next diluted to 100 cc. with distilled water, centrifuged, and the supernatant fluid withdrawn to be used for the carbohydrate and ammonia determinations. These latter determinations on the supernatant were run exactly as outlined for the analogous work in which carbohydrate broth was employed, reported in a previous paper (1930).

4. The sediment contained in the centrifuge tube was resuspended in distilled water and recentrifuged five successive times to remove all minerals, then transferred to accurately weighed crucibles. Subsequently the crucibles were placed in a desiccator over concentrated sulphuric acid and dried to constant weight. The weight of the growth was thus determined.

The calculations were made as for the analagous work previously reported.

RESULTS

The results of one series of determinations are reported in table 1. Three cultures out of the series noted in the table were contaminated by a mold on the termination of the incubation. None of the controls, eight of which were used in these determinations, were contaminated. The determinations here reported typify the results obtained regardless of the duration of incubation or the percentage of the carbohydrate contained within the medium.

It will be noted that in the incubation period reported most of the glucose was utilized by all the Mycobacteria tested. The average glucose content of the control tubes as determined by the Shaffer-Hartman method was 0.926 per cent or 92.6 mgm. of glucose per 10 cc. of media. One hundred and thirty-six milligrams

TABLE 1

Results of quantitative determinations on synthetic media cultures

ORGANISMS	INCUBATION	CARBON DIOXIDE RECOVERED	GLUCOSE UTILIZED	CARBON DIOXIDE POSSIBLE	POSSIBLE CARBON DIOXIDE RECOVERED	AVERAGE OF POSSIBLE CO_2 RECOVERED	TITRATABLE ACIDITY IN CC. N/40 NaOH	NH_3 DECREASE IN TERMS OF N/40 NaOH	WEIGHT OF ORGANISMS	CARBON IN ORGANISMS (CALCULATED)	CARBON RECOVERED AS CO_2	CARBON IN UNUTILIZED CARBOHYDRATE	TOTAL CARBON ACCOUNTED FOR	TOTAL CARBON PRESENT IN BEGINNING
	days	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Myco. tuberculosis (hominis)	42	115	92.6	136	84.5		3.2	4.0						
	42	126	92.6	136	92.6		4.1	4.4						
	42	109	89.6	132	82.8	86.6	4.0	4.4	32.2	16.1	95.2	1.2	112.5	111.3
Myco. berolinensis	42	92	85.1	125	74.4		3.6	3.6						
	42	89	79.1	116	76.5		4.0	4.4						
	42	131	85.9	126	103.0	84.9	3.7	4.0	32	16	85.0	10.1	111.1	111.3
Myco. friburgense	42	99	80.1	118	84.0		3.7	4.4						
	42	113	92.6	136	83.1		3.7	3.6						
	42	124	62.6	136	91.1	86.1	3.9	4.0	33.3	16.7	91.6	5.0	113.3	111.3
Myco. stercusis	42	117	92.6	136	86.0		3.4	4.4						
	42	117	92.6	136	86.0	86.0	4.0	3.2	24.8	12.4	63.8	0.0	76.2	74.2
	42	Contaminated												
Myco. phlei	42	115	90.6	133	85.5	85.5	3.9	4.0	9.1	5.6	31.2	0.7	37.5	37.1
	42	Contaminated												
	42	Contaminated												
B. proteus	24	6.6	14.5	21	31.0	31.0	5.0	0.0						
Controls (average)	42	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	37.1		37.1

(92.6 multiplied by the factor 1.47) of carbon dioxide could have been produced from this amount of glucose were the carbon quantitatively converted to carbon dioxide. While the percentage of possible carbon dioxide recovered varies among the individual cultures of any one organism the averages as given in the column "average per cent of possible carbon dioxide recovered" are comparable.

The titratable acidity in cc. of N/40 sodium hydroxide in every case can be accounted for wholly on a basis of the ammonia decrease. It is to be remembered that the ammonia determination was run on but one fourth of the culture. An error of 0.1 cc. in the titrations would thus introduce an error of 0.4 cc. in the column "ammonia decrease in terms of cc. N/40 NaOH." Since ammonia constituted the only source of nitrogen in the media a decrease would be expected. As the ammonia is withdrawn from the compound ammonium sulphate free sulphuric acid would remain, thus accounting for the change in the reaction of the media towards increased acidity. These results demonstrate indirectly but rather conclusively that no cleavage products of the carbohydrate, which in any way tend to lower the pH, accumulate in the medium.

The composite weight of the organisms from all the cultures of each species of organism was determined. From the work of previous investigators (Kruse (1910)) it would appear that approximately 50 per cent of the weight of washed, dried acid-fast organisms is carbon. Employing this percentage the carbon contained in the organisms was approximated. In the next column in the table the mgm. of carbon recovered as carbon dioxide is given. This represents the total carbon dioxide recovered multiplied by $\frac{3}{11}$. In the next column the carbon remaining in the media as unutilized glucose is given. The total carbon accounted for is listed next, followed by the total carbon present in the uninoculated controls. It will be observed that the latter two figures in each case compare within at least 2 mgm. It would seem that this quite conclusively demonstrates that the carbon in the glucose is converted quantitatively into carbon dioxide or utilized in the synthesis of the bacterial cell. These

results definitely contraindicate the existence of any cleavage products of the glucose in the media.

The results obtained from determinations on one culture of *B. proteus* are given at the end of the table. These results are typical of those obtained with organisms of the colon-typhoid group and of staphylococci. It will be noted that only 6.6 mgm. of carbon dioxide were recovered while 14.5 mgm. of glucose were utilized from which 21.3 mgm. of carbon dioxide could have been produced. Thus 31 per cent of the possible carbon dioxide was recovered as compared to the 85 per cent in the case of the Mycobacteria. Also of significance is the recorded titratable acidity equivalent to 5.0 cc. of N/40 NaOH without any associated demonstrable ammonia decrease, which definitely indicates acid production from the carbohydrate. The growth of these organisms was so limited that a determination of the weight of the growth was not feasible.

DISCUSSION

The above work adds definite evidence to the conception that when organisms of the genus Mycobacterium utilize carbohydrates there is no associated accumulation of cleavage products in the media. The question of the process by which the utilization is accomplished is not clarified by the present work. It is significant that acetic and lactic acids are utilized possibly more readily than glucose (see preceding article). It is thus possible that these acids may be intermediate products in glucose metabolism and that they actually are present outside the cell but that at any given time they are present in such minute amounts as not to be demonstrable by the methods employed. Experiments employing "resting" organisms similar to those devised by J. H. Quastel (1928) and by Kendall et al. (1930) offer an excellent opportunity for further study of the mechanism of utilization of carbohydrates by these organisms.

SUMMARY

1. In glucose synthetic media cultures of the organisms of the genus Mycobacterium studied, all the carbon available in the

media was accounted for as carbon dioxide plus the carbon contained in the organisms, plus that remaining as unutilized carbohydrate.

2. The reaction changed toward increased acidity, accompanying growth of the Mycobacteria in the synthetic medium used. This reaction change can be accounted for completely on a basis of the removal of ammonia from the media. There is no evidence that any cleavage products of the carbohydrates accumulate in the media.

3. It would appear from all evidence at hand that the Mycobacteria utilize carbohydrates completely, no cleavage products accumulating in the media, the carbohydrate being oxidized completely to carbon dioxide and water.

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