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Metabolism of Pathogenic Bacteria Growing Under Aerobic Conditions in Carbohydrate-rich Culture Media.*

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The object of this paper is to show that the carbohydrate metabolism of *rapidly growing* bacteria in meat infusion-peptone culture medium, despite aerobic conditions, is almost entirely anaerobic. Since the culture medium contains most of the constituents of tissues

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TABLE I.
The results are expressed as mM or cc *N* per liter.

Flask	Cultural conditions	Glucose metabolized ×2 mM C_3	Lactic acid		Volatile acids mM	Undetermined non-volatile acidity cc *N*
			Yield mM	Yield %		
I	Anaerobic	32.4	28.1	87	4.5	1.4
II	Aerobic; 100 cc in Erlenmeyer flask	33.8	28.7	85	3.2	.8
III	Aerobic; thin layer in Kolle flask	33.0	27.7	84	3.7	.2

and since the metabolism is predominantly of carbohydrate, these experiments indicate the nature of the metabolic processes of rapidly growing bacteria in tissues. Representative data from 3 pathogens are given; these, as will be shown in a later paper, represent the products and the reactions most characteristic for pathogenic microorganisms as a group.

Freshly prepared, warm beef infusion to which had been added 1% of peptone, 0.7% of $Na_2HPO_4 . 12\ H_2O$, 1% of glucose and 2% by volume of serum, was inoculated with a virulent, rapidly growing, Type I pneumococcus. Measured volumes were transferred to sterile flasks as follows: I, 200 cc into a 200 cc volumetric flask, then immediately covered with oil; II, 100 cc into a 300 cc Erlenmeyer flask; III, 50 cc into a Kolle flask. The Kolle flask was immediately placed on its side and was not thereafter disturbed. Since the openings of flasks II and III and the volume of air above the medium were large, it was necessary to correct for the moisture loss by blanks.

Growth was rapid; it was about 90% complete in 8 hours. The cultures were acidified after about 24 hours of incubation at 37.5°C. They were then analyzed according to the procedures previously described.[1] The results, corrected for moisture loss, are shown in Table I.

Except for the small volume of dissolved oxygen initially present and traces of oxygen which may have entered subsequently through the oil, the condition in flask I may be considered anaerobic. Based upon the sugar consumed, the yield of lactic acid was 87%. This agrees with the results obtained by Hewitt.[2] Almost identical yields of lactic and volatile acids were obtained from flask II, the Erlenmeyer flask in which the conditions were aerobic. Again, from flask III, the Kolle flask which contained a thin layer of culture

1 Friedemann, T. E., *J. Bact.*, 1938, **35**, 527.

2 Hewitt, L. F., *Biochem. J.*, 1932, **26**, 464.

medium, the results agreed with those from the other flasks. The lactic acid yield was 84%, which was practically within the limit of error of the method.

Further evidence of the anaerobic character of the metabolism of pneumococci under relatively aerobic conditions can be seen in the results given in Table II in a previous paper.[1] In experiment 3, for example, 57.1 mM per 1 of glucose x 2 were metabolized and 25.3 mM of lactic acid‡ were produced. Other metabolic products were: 25.2 mM of formic acid, 12.2 mM of acetic acid, and 12.2 mM of ethyl alcohol. The increase of CO_2 was insignificant; less than 0.1 mM in 24 hours. The yield of formic and acetic acids and alcohol was approximately in the ratio of 2 to 1 to 1.§

This is in keeping with the theories of Virtanen, Karström, and Turpeinen,[3] Meyerhof and Kiessling,[4] and Neuberg and Kobel.[5] According to these theories, glucose, through triose, finally yields 1 mole each of glycerophosphate and phosphopyruvate. These are further metabolized as follows:

Glycerophosphate → C_2H_5OH + HCOOH[6]
Phosphopyruvate → CH_3COOH + HCOOH[7]

The calculated yield of products according to these anaerobic reactions was realized in this experiment. The small yield of CO_2 is further evidence of the anaerobic character of the metabolism.

The same maximum yield of approximately 2 moles of formic acid for each mole of acetic acid or alcohol can often be obtained from other bacteria when the growth is very rapid. The rapid acidification of the medium due to growth in the presence of an excess of sugar, appears to protect the formic acid from decomposition. Examples

‡ The yield of lactic acid, 44.3% in this experiment, illustrates the wide variations which may be obtained in the same culture medium under apparently the same conditions. The lactic acid yield is not always as constant as indicated by Hewitt.[2]

§ Lactic acid is obtained from actively growing pneumococci, even when the culture medium is exposed to air in rapidly moving thin layers. 50.0 cc of inoculated culture medium were introduced into a sterile 18 l bottle. The bottle was rapidly rotated during a period of 8 hours. The results, expressed as mM per liter, were as follows: sugar × 2 consumed, 22.3; lactic acid, 10.5; acetic acid, 11.1; CO_2, 11.0; undetermined non-volatile acidity, —1.1. *The absence of alcohol should be noted.* The lactic acid yield was 47%.

3 Virtanen, A. I., Karström, H., and Turpeinen, O., *Naturwissenschaften*, 1929, **17**, 877. More complete discussion is given in *Z. physiol. Chem.*, 1930, **187**, 7.

4 Meyerhof, O., and Kiessling, W., *Biochem. Z.*, 1933, **264**, 40; **267**, 313.

5 Neuberg, C., and Kobel, M., *Biochem. Z.*, 1934, **272**, 445.

6 Harden, A., *Proc. Chem. Soc.*, 1901, **17**, 57.

7 Neuberg, C., and Ringer, M., *Biochem. Z.*, 1915, **71**, 237.

TABLE II.

Growth 12 hours at 37.5°C in meat extract medium which contained 1% of Witte peptone, 1.8% of $Na_2HPO_4 \cdot 12H_2O$, and 1% of glucose. Results are expressed as mM or cc *N* acid per l.

Organism	Glucose × 2 used mMC_3	Products					
		Lactic acid mM	Formic acid mM	Acetic acid mM	Ethyl alcohol mM	Undetermined non-volatile acidity cc *N*	Lactic acid yield %
Eberthella typhosa	39.4	4.1	28.8	15.4	13.1	0.8	10.4
Escherichia coli	51.8	13.9	27.2	14.1	14.0	3.2	26.8

of such results|| are shown in Table II. The absence of succinic acid in this experiment, as indicated by the "undetermined non-volatile acidity," should be noted.

It is interesting to note that Grey and Young[8] obtained equal or higher yields of lactic acid from *Es. coli* in those flasks which contained oxygen above the surface of the medium. Growth, according to Grey, was most rapid in these flasks. Hewitt[2] found identical yields of lactic acid from pneumococci in tubes of culture media kept under anaerobic conditions and in similar tubes exposed to air.

These results are significant because they clearly indicate the nature of the metabolic processes of bacteria growing in tissues. Strictly anaerobic conditions are perhaps never encountered in normally functioning tissues. Such conditions may be approached in edematous traumatized areas with marked stasis or occlusion of blood vessels. It is no doubt realized in the purulent exudate in the alveoli during lobar pneumonia[9] and in the interior of abscesses. Most organisms, however, in the early stages of an infection, encounter relatively aerobic conditions. These are comparable to the limited aerobiasis obtaining in test tube or Erlenmeyer flask cultures. Our results from carbohydrate-rich culture media, under these relatively aerobic conditions are almost identical with those obtained under strictly anaerobic conditions. Despite the complexity of the culture medium, carbohydrate is the chief source of energy. The metabolic products are relatively few in number, and the volatile products may be obtained in the proportions indicated by the reactions shown above.

|| The yield of lactic acid in this experiment was lower and the yield of volatile products was higher than reported by previous investigators. All of the published data represent the activities of these 2 organisms after from one to 2 weeks of growth in a medium consisting only of peptone, dextrose, and $CaCO_3$.

[8] Grey, E. C., and Young, E. G., *Proc. Roy. Soc. London*, 1921, **92** B, 135.

[9] Friedemann, T. E., and Graeser, J. G., *J. Exp. Med.*, 1937, **67**, 481.

Tissues contain considerable quantities of metabolizable carbohydrate which consist of free sugar, glycogen and other polysaccharides. These sugars are readily metabolized by many pathogenic microorganisms (unpublished data) when added to culture medium or serum; the products in every instance have been the same as are obtained from glucose.

Summary and Conclusions. *Diplococcus pneumoniæ, Eberthella typhosa,* and *Escherichia coli* were grown 24 hours at 37.5°C in meat infusion or meat extract culture medium enriched with 1% of peptone, 0.7 to 1.8% of $Na_2HPO_4 . 12\ H_2O$, and 1% of glucose. The pneumococcus was grown under anaerobic as well as highly aerobic conditions. The principal products were lactic acid, formic acid, acetic acid, and ethyl alcohol. The last 3 products appeared in the approximate ratio of 2 to 1 to 1. The reactions representing their formation most likely are: Glucose → hexose diphosphate → 2 triosephosphate → glycerophosphate and phosphoglycerate. Glycerophosphate → C_2H_5OH + HCOOH. Phosphoglycerate → phosphopyruvate → CH_3COOH + HCOOH. These are anaerobic mechanisms. Only a trace of CO_2 was obtained from pneumococci.

Rapidly growing bacteria may metabolize sugar almost entirely by anaerobic mechanisms when grown under relatively aerobic conditions. The same intermediary reactions are probably also utilized by microörganisms when growing in tissues in the course of an infection.

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Occurrence of Sporadic Bacillemia in Experimental Tuberculosis in Dogs.

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Wilson[1] in summarizing the work on bacillemia in human tuberculosis up to 1933, concludes that bacillemia, detectable by present methods, is, except as a transitory phenomenon, rarely present until the disease has assumed an acute phase. Because of this discrepancy between the reported rare occurrence of bacillemia and the common demonstration of hematogenous tuberculous lesions at necropsy, it

[1] Wilson, G. S., *Tuberculous Bacillemia,* London, 1933.