THE BREAKDOWN OF CARBOHYDRATES BY *ASTEROCOCCUS MYCOIDES*, THE ORGANISM OF BOVINE PLEUROPNEUMONIA

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Summary

Cell suspensions of *A. mycoides* oxidized glucose, fructose, and mannose. Glucose, pyruvate, lactate, and, in the presence of catalase, glycerol were oxidized quantitatively to acetate and carbon dioxide. Anaerobically, pyruvate underwent dismutation to acetate, lactate, and carbon dioxide. Neither glucose nor glycerol were attacked anaerobically by intact cell suspensions. No other pentoses, hexoses, di-, tri-, or polysaccharides, or polyhydric alcohols, were attacked by cells grown in a complex undefined medium.

None of the following substances was attacked either by intact cell suspensions or by suspensions treated by freezing and thawing: formate, acetate, succinate, fumarate, malate, citrate, aspartate, glutamate, α-ketoglutarate, alanine, or proline. It is concluded that the organism possesses no Wood-Werkman system, or di- or tricarboxylic acid cycle for the oxidation of these substances.

Evidence was obtained that the organism lacks a cytochrome system, but possesses catalase. Growth in a complex medium was almost absent under anaerobic conditions and was greatly increased by aeration, but although strongly aerobic in its growth requirements, *A. mycoides* resembles in its metabolism the facultative organisms rather than the strict aerobes.

I. INTRODUCTION

The organism of bovine pleuropneumonia (*Asterococcus mycoides*) and the "pleuropneumonia-like" organisms isolated from other sources, e.g. from mice, human and bovine genital tracts, sheep, goats, and chick embryo, form a group of closely related species possessing many similarities to the L forms of bacteria (Tulasne 1951; Dienes and Weinberger 1951).

Virtually nothing, however, has yet been published concerning the intermediary metabolism of any of the organisms belonging to this group, or of bacteria in the L phase. Dujardin-Beaumetz (1900) identified acetic acid as the sole volatile acid produced in cultures of *A. mycoides* in media containing fermentable carbohydrate. Holmes and Pirie (1932) showed the presence of lactic dehydrogenase in resting cell suspensions, but did not obtain methylene blue reduction with glucose, formate, succinate, alanine, or hypoxanthine as substrates. Similar results were reported by Warren (1942). Recently, Somerson and Morton (1953) have interpreted the accumulation of formazan deriva-

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tives in surface colonies of pleuropneumonia-like organisms on media containing tetrazolium salts as evidence for the presence of flavoprotein oxidases.

In this paper, the results of a general survey of the overall catabolic reactions catalysed by *A. mycoides* are described. It is hoped later to extend the observations to include some L forms of bacteria and their parent organisms.

II. Methods and Materials

(*a*) *Cultural Methods*

(i) *The Organism.*—Strain "V5" was isolated from a case of acute bovine contagious pleuropneumonia in 1936, and has since been used for the preparation of prophylactic vaccine (Campbell 1938).

(ii) *Medium.*—Cells were harvested from cultures in BVF-OS medium (Turner, Campbell, and Dick 1935). It is essentially a pig-stomach digest of beef liver and muscle, buffered with M/15 phosphate at pH 7.4. Ox serum (10 per cent. v/v) is added before sterilizing the medium by filtration through Seitz pads.

(iii) *Growth Conditions.*—Cells were grown on 2 l. of medium in 5-l. florence flasks fitted with rubber bungs through which passed a filling and inoculating tube and a second tube terminating in a sintered glass disk of porosity 4. On inoculating with 2 ml of a 3-4 day culture, oxygen containing 5 per cent. carbon dioxide was passed through the sintered glass disk at an approximate rate of 150 ml/hr during incubation at 37°C for 40 hr. An electrically heated nichrome wire in the neck of the flask prevented froth reaching the cotton-wool plugs.

Cultures grown under these conditions consisted mainly of small forms appearing as points of light by microscopic examination with dark ground illumination, and occasional short filaments and discules.

(*b*) *Preparation and Standardization of Cell Suspensions*

(i) *Preparation of Suspensions.*—The cells were recovered from the cultures by passage through a steam-driven Sharples supercentrifuge (62,400*g* at periphery of cylinder). The cylinder was lined with a plastic parchment-like material, from which the cell material was scraped off and resuspended in distilled water. The cells were not washed, since endogenous respiration of unwashed cells during the usual manometric experiments was low, and washing was found to decrease the rate of oxidation of all substrates tested. Cell suspensions were used within 6 hr of their preparation. The activity of suspensions was not increased, nor the loss of activity after storage decreased, by suspending the cells in Krebs-Ringer solutions, saline, or buffers instead of distilled water.

(ii) *Standardization of Cell Suspensions.*—In most experiments optical density measurements were used, since it was found that, for suspensions prepared

from cells grown under standard conditions, cell nitrogen and dry weight were related to the optical density of suspensions in water. In some experiments, the quantitative Biuret reaction (Stickland 1951) was used.

(iii) *Preparation of Frozen and Thawed Suspensions.*—Suspensions were rapidly frozen in a "dry-ice"-alcohol bath and allowed to thaw. The pH was maintained between 6.5 and 7.0 during thawing by the cautious addition of 0.05N sodium hydroxide. The process was repeated twice or thrice. The preparations became extremely mucoid during this treatment.

(iv) *Estimation of Growth in Cultures.*—Samples of the culture were centrifuged for 15 min at 15,000 r.p.m. in the high-speed head of the International centrifuge, the deposit evenly suspended in the same volume of water, and the dry weight determined from optical density measurements with a filter transmitting maximally at 440 mμ.

(c) *Analytical Methods*

(i) *Manometric Methods.*—Conventional Warburg manometers were used. Oxygen uptake as measured by the direct method of Warburg, and by the diethanolamine-carbon dioxide buffer method as modified by Krebs (1951) showed that the absence of carbon dioxide did not influence the oxygen uptake with glucose, lactate, or pyruvate as substrates. In all subsequent experiments the direct method of Warburg was used for measuring oxygen uptakes. Carbon dioxide evolution was measured after tipping in acid at the end of the experimental period. The experiments were carried out at a temperature of 37°C.

(ii) *Carbon Balance Experiments.*—Non-gaseous products were estimated in the contents of the manometer vessels. The contents of several flasks were pooled to obtain sufficient material for the estimations. Trichloracetic acid was used to deproteinize the material for pyruvate and lactic acid estimations. Samples for estimation of volatile acid and of acetoin (after oxidation to diacetyl) were steam-distilled in the Markham apparatus without previous deproteinization.

(iii) *Pyruvic Acid.*—Pyruvic acid was estimated by the method of Friedemann and Haugen (1943).

(iv) *Acetic Acid.*—Acetic acid was determined by the method of Friedemann (1938), and in some experiments, its identity was confirmed by partition chromatography on silica gel columns (Elsden 1946).

(v) *Lactic Acid.*—Lactic acid was estimated by the colorimetric method of Barker and Summerson as described by Umbreit, Burris, and Stauffer (1949).

(vi) *Acetoin.*—Acetoin was oxidized to diacetyl by excess ferric chloride (van Niel 1927), and estimated colorimetrically by the method of White, Krampitz, and Werkman (1946).

(*d*) *Materials*

(i) *Pyruvic Acid.*—Pyruvic acid was prepared by double distillation of commercial pyruvic acid as described by Lipschitz, Potter, and Elvehjem (1938), and used with the precautions concerning dilution and neutralization recommended by these authors.

(ii) *Coenzyme Concentrate.*—The mixed nucleotide "fraction *A*," prepared from fresh pig liver as described by Le Page and Mueller (1949) for the preparation of triphosphopyridine nucleotide, was used as a source of coenzymes. The preparation was assayed for coenzyme *A* activity by the method of Handschumacher, Mueller, and Strong (1951). Although not assayed for other coenzymes, similar preparations have been found to contain appreciable amounts of both di- and triphosphopyridine nucleotides, pyridoxal phosphate, cocarboxylase, and bound biotin, in addition to coenzyme *A* (Buyske *et al.* 1951).

(iii) *Catalase.*—Beef liver catalase was prepared by the method of Mosimann (1951).

(iv) *Diphosphopyridine Nucleotide.*—This was prepared by the method of Williamson and Green (1940), and its purity assayed enzymically with crystalline alcohol dehydrogenase prepared by the method of Racker (1950).

III. Experimental

(*a*) *Breakdown of Sugars, Polysaccharides, and Sugar Alcohols*

(i) *Substrates Attacked.*—The ability of cell suspensions to oxidize the sugars, polysaccharides, and sugar alcohols commonly used in determinative bacteriology was tested manometrically in phosphate buffer at pH 7.4. An oxygen uptake was found with glucose, mannose, and fructose. The rates, expressed as Q_{O_2} (N) for these three hexoses with suspension from the same batch of cells, were 350, 330, and 130 respectively. Glycerol was attacked at a high initial rate, but the oxygen uptake had almost ceased within 15 min of adding the substrate. In the presence of catalase, oxygen uptake continued at a constant rate corresponding to a Q_{O_2} (N) of 360 (Fig. 1). No other pentoses, di-, tri-, or polysaccharides, or sugar alcohols tested were attacked by cells grown in BVF-OS medium. The pH had little effect on the rate of glucose oxidation over the range 7-8; the rate decreased to about half this value at a pH of 8.8.

(ii) *Fermentation of Glucose and Glycerol.*—The ability of cell suspensions to form acid from glucose and glycerol was tested in bicarbonate buffer with 5 per cent. carbon dioxide in nitrogen as the gas phase. None was formed from either substrate, although the suspension oxidized glucose vigorously when tested aerobically in phosphate buffer. The formation of lactic acid from glucose anaerobically was also tested in phosphate buffer with oxygen-free nitrogen as the gas phase. Lactic acid was estimated in the flask contents after 60 min

incubation. Lactic acid formation was negligible and less than in the control flask without glucose.

(iii) *Products of Glucose Oxidation.*—The results obtained in four separate experiments for the oxidation of glucose by cell suspensions in phosphate buffer are shown in Table 1. Acetate accumulated as the end-product of the oxidation. Carbon recovery was complete. The figures agree with the equation for the reaction:

$$C_6H_{12}O_6 + 2O_2 \longrightarrow 2CO_2 + 2CH_3COOH + 2H_2O.$$

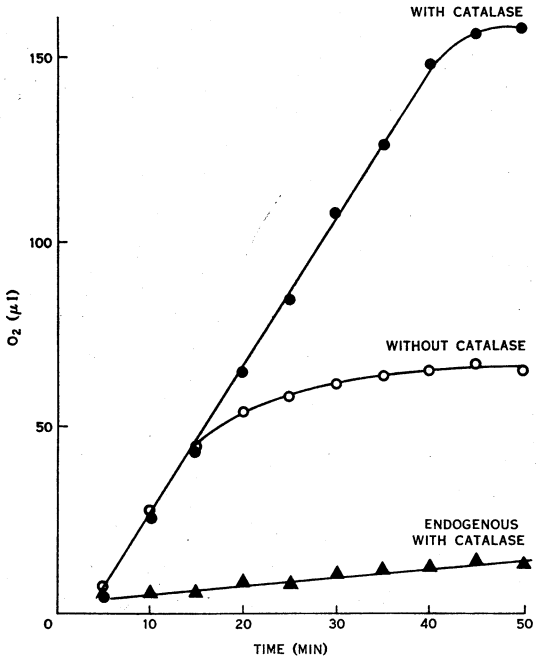

Fig. 1.—Effect of catalase on glycerol oxidation by suspensions of *A. mycoides*. Warburg vessels contained: M/5 phosphate buffer, 1.45 ml; bacterial suspension, 0.5 ml ≡ 0.68 mg N (main compartment); catalase solution, 0.25 ml (side bulb 1); M/20 glycerol, 0.1 ml (side bulb 2); 20 per cent. w/v KOH, 0.2 ml (centre well).

(iv) *Products of Glycerol Oxidation.*—The products of the oxidation of glycerol in the presence of catalase are shown in Table 2. Carbon recovery in the products was again complete, and the figures agree with the equation:

$$C_3H_8O_3 + 1.5O_2 \longrightarrow CO_2 + CH_3COOH + 2H_2O.$$

(*b*) *Breakdown of Pyruvate and Lactate*

(i) *Pyruvate Oxidation.*—The cell membrane of the organism appears to be permeable to pyruvate, since it was oxidized at a rate comparable with that

found with other substrates, and the rate of oxygen uptake did not differ over a range of pyruvate concentrations of 0.5-0.01M. Lower pyruvate concentrations were not tested.

TABLE 1

PRODUCTS OF GLUCOSE OXIDATION BY *A. MYCOIDES*

Warburg vessles contained: M/5 phosphate buffer (pH 7·4) 1·5 ml; bacterial suspension 0·5 ml; M/40 glucose 0·2 ml (5μM)

Values in μM

Experiment	Glucose Oxidized	Oxygen	Carbon Dioxide	Acetate
1	5	9·8	9·8	9·65
2	5	9·4	10·3	11·35
3	5	9·95	10·9	8·4
4	5	8·75	9·9	10·9
Mean	5	9·5	10·25	10·1
mg C	0·36		0·123	0·242
% C recovered = 100				

The products for pyruvate oxidation are shown in Table 3. The figures agree with the equation:

$$CH_3CO.COOH + \tfrac{1}{2}O_2 \longrightarrow CO_2 + CH_3COOH.$$

TABLE 2

PRODUCTS OF GLYCEROL OXIDATION BY *A. MYCOIDES*

Warburg vessels contained: M/5 phosphate buffer (pH 7·4) 1·2 ml; bacterial suspension 1·0 ml; catalase solution 0·3 ml; M/20 glycerol 0·2 ml (10μM)

Values in μM

Glycerol Oxidized	Oxygen	Carbon Dioxide	Acetate
10	14·3	9·2	10·0
mg C 0·36		0·110	0·240
% C recovered = 97			

(ii) *Pyruvate Dismutation.*—Pyruvate was also attacked anaerobically. The results obtained in two separate experiments for the anaerobic breakdown of pyruvate in phosphate buffer, with oxygen-free nitrogen as the gas phase, are shown in Table 4. Recovery of carbon in the products in these experiments was only 87 per cent. Lactate and acetate were found in approximately equimolar amounts, and carbon dioxide in slightly greater amounts. The figures are

consistent with the breakdown of pyruvate by the dismutation reaction, together with the formation of a small amount of acetoin:

$$2CH_3CO.COOH + H_2O \longrightarrow CH_3COOH + CH_3CHOH.COOH + CO_2$$
$$2CH_3CO.COOH \longrightarrow CH_3CHOH.COCH_3 + 2CO_2.$$

The formation of small amounts of acetoin during the dismutation reaction was shown subsequently.

TABLE 3

PRODUCTS OF PYRUVATE OXIDATION BY *A. MYCOIDES*

Warburg vessels contained: M/5 phosphate buffer (pH 7·4) 1·0 ml; bacterial suspension 0·4 ml; M/10 pyruvate 0·2 ml (20μM)

Values in μM

Pyruvate Oxidized	Oxygen	Carbon Dioxide	Acetate
20	9·1	18·0	19·4
mg C 0·72		0·216	0·466
% C recovered = 95			

TABLE 4

PRODUCTS OF ANAEROBIC BREAKDOWN OF PYRUVATE BY *A. MYCOIDES*

Warburg vessels contained: M/5 phosphate buffer (pH 7·4) 1·25 ml; M/10 pyruvate 0·2 ml (20μM); bacterial suspension 0·5 ml

Values in μM

Experiment	Pyruvate Disappearing	Carbon Dioxide	Lactate	Acetate
1	20	10·0	8·06	8·34
2	20	9·9	9·02	8·42
Mean	20	9·95	8·54	8·38
mg C	0·72	0·12	0·308	0·200
% C recovered = 87				

(iii) *Oxidation of Lactate.*—Methylene blue reduction was relatively rapid in Thunberg tube experiments with lactate as substrate compared with other substrates tested. In one experiment, for example, the times for 90 per cent. decolourization were 1, 7, and 15 min with lactate, glucose, and pyruvate respectively. The products for the oxidation of DL-lactate are shown in Table 5. The figures agree with the equation:

$$CH_3CHOH.COOH + O_2 \longrightarrow CH_3COOH + CO_2 + H_2O.$$

Slightly less than half was oxidized, whereas a sample of L-lactate was wholly oxidized.

(*c*) *Oxidation of Tricarboxylic Acid Cycle Intermediates*

The ability of the organism to metabolize some of the intermediate compounds of the tricarboxylic acid cycle, and also the amino acids which may be converted by deamination or transamination to tricarboxylic acid cycle intermediates, was tested both in Thunberg tube experiments with methylene blue as electron acceptor, and in manometric experiments in which oxygen uptake was measured. Intact cell suspensions, as well as suspensions treated by freezing and thawing to break down permeability barriers, were tested. The following substances were not attacked in any of these experiments: citrate, malate, succinate, fumarate, formate, acetate, glutamate, aspartate, α-ketoglutarate, proline, or alanine. The ability of suspensions treated by freezing and thawing to oxidize acetate, malate, citrate, succinate, and glutamate was also tested manometrically in bicarbonate buffer with 5 per cent. carbon dioxide in nitrogen as the gas phase. Methylene blue, which can replace oxygen as electron acceptor for the oxidation of glucose, was added as electron acceptor in this experiment. The pig-liver preparation "fraction *A*" was used as a source of coenzymes. The manometer vessels contained 1.25μM of glucose, or 2.5μM of the other substrates, 8μM methylene blue, 1 mg of the "fraction *A*" preparation, and suspension equivalent to 5 mg nitrogen. The evolution of carbon dioxide from glucose was very rapid and was almost complete within 45 min, whereas with the other substances it was very slow and was the same as that without substrate.

TABLE 5

PRODUCTS OF LACTATE OXIDATION BY *A. MYCOIDES*

Warburg vessels contained: M/5 phosphate buffer (pH 7·4) 1.5 ml; bacterial suspension 0·5 ml; M/10 DL-lactate 0·2 ml (20μM)

Values in μM

Initial Lactate	Final Lactate	Lactate Oxidized	Oxygen	Carbon Dioxide	Acetate
20 mg C	11·2	8·8 0·317	8·54	8·54 0·102	8·55 0·205
% C recovered = 97					

(*d*) *Terminal Respiratory Enzymes*

(i) *Cytochrome System.*—It was concluded from the following evidence that the organism lacks a cytochrome system.

(1) The rate of glucose oxidation was not very sensitive to inhibition by cyanide, a concentration of 0.46×10^{-3}M caused only a 30 per cent. inhibition of the rate. Pyruvate oxidation was relatively insensitive to inhibition by azide; concentrations of 10^{-2}M and 10^{-3}M inhibited the rate of oxygen uptake by 60 per cent. and 20 per cent. respectively.

(2) Cytochrome bands could not be detected upon spectroscopic examination, even after freezing in liquid air.

(3) Cell suspensions were incapable of oxidizing *p*-phenylene diamine when tested manometrically.

TABLE 6

GASEOUS REQUIREMENTS FOR GROWTH OF *A. MYCOIDES* IN BVF-OS MEDIUM

50-ml volumes of medium in 250-ml erlenmeyer flasks; liquid depth approximately 1 cm. For deep culture 50 ml medium in narrow bottle; liquid depth approximately 7 cm. Inoculum: 0·02 ml culture. — = No detectable growth

Gas Phase	Growth (mg dry wt./ml culture)	
	40 Hr	64 Hr
H_2	—	0·05
N_2	—	0·075
O_2	—	0·063
38 mm CO_2; 722 mm H_2	—	0·14
44 mm CO_2; 5 mm O_2; 711 mm N_2	0·045	0·215
53 mm CO_2; 140 mm O_2; 567 mm N_2	0·19	0·292
40 mm CO_2; 720 mm O_2	0·225	0·425
Air (liquid depth 1 cm)	0·115	0·385
Air (liquid depth 7 cm)	0·06	0·135

(ii) *Gaseous Requirements for Growth in BVF-OS Medium.*—To test the effect of the oxygen and carbon dioxide tensions in the gas phase on growth of the organism in BVF-OS medium, 250-ml erlenmeyer flasks containing 50-ml volumes of medium were inoculated with 0.02 ml of culture. The flasks were incubated in closed jars which were evacuated and filled (twice) with the appropriate gas mixture. In addition one flask was exposed to the air of the incubator, and also one narrow bottle containing the same volume of medium, but having a liquid depth of approximately 7 cm. Residual oxygen in the gas phase of the jars containing hydrogen or hydrogen-carbon dioxide gas mixtures was removed by combustion. Samples of the cultures were taken after 40 and 64 hr incubation for estimation of growth. The results are recorded in Table 6. It may be seen that growth was scanty and delayed in the absence of oxygen; growth was also very slight with pure oxygen as the gas phase. Addition of carbon dioxide to the gas phase allowed more growth under anaerobic conditions. With a carbon dioxide tension in the gas phase varying in different jars from 38 to 53 mm, growth was then limited by the partial pressure of oxygen, being heaviest with a partial pressure of 720 mm.

(iii) *Methylene Blue and Ferricyanide as Final Electron Acceptors for the Oxidation of Glucose.*—As stated above, intact cell suspensions were incapable of fermenting glucose or glycerol. The ability of methylene blue or ferricyanide

to replace oxygen as final electron acceptor was therefore tested in bicarbonate buffer with 5 per cent. carbon dioxide in nitrogen as the gas phase. With glucose as substrate, carbon dioxide was evolved at approximately the same rates in the presence of either acceptor. Methylene blue could not replace oxygen

TABLE 7

METHYLENE BLUE AS ELECTRON ACCEPTOR IN GLUCOSE OXIDATION BY *A. MYCOIDES*: EFFECT OF METHYLENE BLUE CONCENTRATION

Thunberg tubes contained: bacterial suspension 0·5 ml; M/5 phosphare buffer 1·0 ml; methylene blue solution (stopper) 0·5 ml; M/100 glucose (stopper) 0·1 ml (1·0 μM)

Methylene Blue (μM)	Decolourization Time (min)
1·6	5
2·0	6
2·4	7·5
2·8	8·0
3·2	9·5
3·6	10
4·0	11·5
4·4	>30
4·8	>30

for the oxidation of glycerol. The oxidation of glucose with methylene blue as electron acceptor in place of oxygen was investigated in more detail. A Thunberg tube experiment, the results of which are set out in Table 7, showed that 4 moles of methylene blue were reduced per mole glucose oxidized. In manometric experiments with bicarbonate-carbon dioxide buffer at pH 7.4, a total of 8 moles of carbon dioxide were evolved per mole glucose oxidized. By measurements of the initial bicarbonate concentration, and the concentration remaining at the end of the reaction, it was shown that of the total of 8 moles of carbon dioxide evolved during the oxidation of 1 mole of glucose in bicarbonate, approximately 6 moles were due to acid formation, and 2 moles were due to the formation of carbon dioxide (Table 8). Clerici fluid was used as the manometer fluid in these experiments in order to measure the large gas evolution. In phosphate buffer, with nitrogen as the gas phase, 2 moles of carbon dioxide were evolved per mole glucose oxidized.

The results of these experiments are consistent with the equation for the oxidation of glucose:

$$C_6H_{12}O_6 + 4MB \longrightarrow 2CH_3COOH + 4MBH_2 + 2CO_2.$$

The reduced form of methylene blue, having a lower pK value than the oxidized form, displaces carbon dioxide from bicarbonate buffer at the pH of these experiments (pH 7.4), although not quantitatively.

DPN could also replace oxygen as electron acceptor for glucose oxidation. This was shown with frozen and thawed suspension in bicarbonate buffer with 5 per cent. carbon dioxide in nitrogen as the gas phase. In the presence of

excess DPN a total of 4 moles of carbon dioxide were evolved per mole glucose oxidized.

(iv) *Catalase.*—Cell suspensions possessed catalase activity. Catalase activity was measured manometrically by oxygen evolution from hydrogen peroxide. A value, calculated from the rate over the first 5 min after tipping in substrate of Q_{O_2} (N) 800 was found at a temperature of 25°C.

TABLE 8

FORMATION OF ACID AND CARBON DIOXIDE IN GLUCOSE OXIDATION BY *A. MYCOIDES*, WITH METHYLENE BLUE AS ELECTRON ACCEPTOR

Warburg vessels contained: glucose 1 μM; methylene blue 5 μM; tested (*a*) in $NaHCO_3$ buffer with 5% CO_2 in N_2 (v/v) in gas phase (pH 7·4); acid estimated by bicarbonate disappearance; (*b*) in phosphate buffer (pH 7·4) with N_2 in gas phase

Values in μM

Experiment	Glucose Oxidized	HCO_3^-–CO_2 Buffer		Phosphate Buffer CO_2
		Acid	CO_2	
1	1·00	5·36	1·92	1·84
2	1·00	5·76	2·00	2·00

IV. DISCUSSION

The failure of earlier workers (Holmes and Pirie 1932; Warren 1942) to detect dehydrogenase activity in washed cells of the organism with glucose as substrate, and the low activities recorded for lactic dehydrogenase, may have been due either to excessive washing of the cells with consequent loss of coenzymes, to the growth conditions used, or to the age of the cells when harvested. In the present work the oxidative activity of the cells was comparatively high. Values for oxygen uptake, expressed as Q_{O_2} (N), of 300-400 were found regularly for the oxidation of all substrates attacked, with the exception of mannose.

Previous workers have investigated the formation of acid from carbohydrates etc. during growth by *A. mycoides.* Dujardin-Beaumetz (1900) found acid formation from glucose, fructose, and maltose. Nakamura, Futamura, and Watanuki (1926) added dextrin; Tang *et al.* (1935) mannose; and Turner, Campbell, and Dick (1935) starch. In the present work it was found that glucose, fructose, mannose, and glycerol were oxidized by cell suspensions, but not maltose, starch, or dextrin. It is possible that the oxidation of the latter substances requires adaptation.

The hexoses attacked (glucose fructose, and mannose), suggest that the initial step in their degradation is a phosphorylation by hexokinase.

Hexokinase is specific for the phosphorylation of these three sugars. Mannose is phosphorylated by yeast hexokinase at a considerably slower rate than are glucose and fructose. The rate of oxygen uptake found for mannose oxidation was considerably slower than that for glucose or fructose oxidation, suggesting that the rate of the initial phosphorylation reaction may limit the rate of mannose oxidation. The pathway for the oxidation of these hexoses, and the reasons for the absence of anaerobic glucose breakdown by intact cells are considered in a later paper (see page 37).

The oxidation of glycerol by a strain of *Streptococcus faecalis* which did not oxidize pyruvate has been investigated by Gunsalus and Umbreit (1945), who obtained evidence for the following mechanism for the oxidation by this organism. Glycerol was phosphorylated, probably to D-glycerophosphate. Glycerophosphate was oxidized by a flavoprotein enzyme reacting directly with oxygen to form D-glyceraldehyde phosphate and hydrogen peroxide. Further oxidation of triose phosphate was through the Embden-Meyerhof scheme to pyruvate, which in this organism was then reduced to lactate in order to regenerate oxidized diphosphopyridine nucleotide. In the absence of a mechanism for decomposing hydrogen peroxide (e.g. added catalase or pyruvate) the reaction ceased before all the glycerol was oxidized. With *A. mycoides* also the addition of catalase was necessary to allow the oxidation to continue. Furthermore, methylene blue could not replace oxygen for this oxidation. It is assumed that the pathway for glycerol oxidation is similar to that found by Gunsalus and Umbreit for *Strep. faecalis,* except that in *A. mycoides* pyruvate is further oxidized to acetate and carbon dioxide.

The overall equation demanded by this pathway:

$$C_3H_8O_3 + 1.5O_2 \longrightarrow CH_3COOH + CO_2 + 2H_2O,$$

agrees with the results obtained in the carbon balance experiment.

The nature of the terminal respiratory enzyme system catalysing the transfer of electrons from the reduced coenzymes to oxygen was not determined. Cytochrome absorption bands could not be detected, and the absence of oxidation of *p*-phenylene diamine—assuming the cell membrane to be permeable to this substance—indicates the absence of cytochrome *c* (Keilin and Hartree 1939). The formation of hydrogen peroxide during glucose, lactate, or pyruvate oxidation was not tested directly, but added catalase was without effect on these oxidations.

The almost complete absence of anaerobic growth may perhaps be explained by the absence of a mechanism for anaerobic glucose fermentation. The only energy-yielding reaction occurring anaerobically so far detected in the organism is the pyruvate dismutation reaction. It is suggested in a later paper that oxygen is required in glucose breakdown for the re-oxidation of reduced DPN. It might be possible to replace oxygen by other electron acceptors for growth purposes also.

The evidence for the absence of any mechanism for the oxidation or metabolism of the intermediate compounds of the Wood-Werkman scheme, or of the tricarboxylic acid cycle, appears conclusive. Acetate accumulated as the end-product of the oxidation of all substrates attacked, and none of the inter-

mediate compounds of these systems was oxidized. That the method used of freezing and thawing the cells results in an alteration or destruction of permeability barriers was evident in experiments to be described later.

As a result of this general survey, it may be said that *A. mycoides*, although strongly aerobic in its growth requirements in the culture medium used, resembles in its metabolism the facultative organisms such as *Strep. faecalis* (strain 10C1) whose metabolism has been described by O'Kane (1950), rather than the strict aerobes. Although oxygen is required for growth, it is doubtful whether the organism derives any additional energy from the final electron transfer to oxygen.

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VI. References

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