Obituary Notice

ERNST FREUND, 1863–1946

Professor Ernst Freund died in London on 2 June 1946 at the advanced age of 83. Born in Vienna, he studied medicine there, obtaining his M.D. degree when he was only 22. In the middle of the nineties of the last century he was appointed Director of the Biochemical Department of the 'Rudolf-Spital', which hospital he served until he reached the retiring age, for part of the time as Professor of Pathological Chemistry in the University of Vienna.

This period of Freund's long life coincided with a great epoch in European and Viennese medical science to which he made many valuable contributions, such as the introduction of sodium citrate as an anticoagulant in blood transfusion (1891), a proposal which arose directly out of his accomplished study of the coagulation of the blood. Many of his discoveries and innovations so readily found acceptance that their authorship has not infrequently been lost sight of. It was his endeavour always to apply chemical technique to the study of pathological problems.

Freund's research work covered a wide field, but among his many interests may be singled out the physiology and pathology of digestion and the mechanism of absorption of nutrients through the intestinal wall, the importance to the organism of a normally balanced bacterial flora in the gut and methods of urine analysis. He devoted much time to the study of tuberculosis, but it was undoubtedly

the problem of malignant growth which exercised for him the greatest fascination.

On this subject he was led in those early days to conclusions, many of which stimulated further work in this very difficult field. With his collaborator G. Kaminer he described the cytolytic reaction which he modified and revised in many respects in later years. The technique of this test was much too complicated to become a routine investigation. It is, however, fair to say that these highly original ideas have stimulated widespread search for a diagnostic reaction for cancer, a search which is still proceeding.

When Freund was approaching the retiring age, the Pearson Cancer Research Foundation was created by Mr F. F. A. Pearson to enable him to continue his work upon malignant disease, which he did until his last day, first at the Rudolf-Spital in Vienna and later in London, when the whole Foundation was transferred to England as a result of the Nazi invasion of Austria.

Freund's long lifetime has witnessed very great changes in biochemical outlook, theory and technical achievement. He belonged to an older generation of biochemists, a pioneer generation, if one may use the term, but his devotion to his subject and intellectual enthusiasm remained with him to the last. He has left to posterity a scientific inheritance of more than 200 papers.

C. R.

The Respiration of *Mycobacterium phlei*

By N. L. EDSON AND G. J. E. HUNTER (Travis Research Fellows),
Travis Laboratory, Medical School, University of Otago, New Zealand

(Received 27 August 1946)

Stephenson & Whetham (1922, 1924) found that *Mycobacterium phlei* growing in a synthetic medium utilized either glucose or lactate as the sole source of carbon. No breakdown product other than CO₂ was detected. Merrill (1930, 1931 *a, b*) likewise found no evidence of partial degradation in cultures. Ebina & Nakamura (1937) studied the oxidation of glucose by washed suspensions, noting the absence of aerobic glucolysis. The measurements of Edson & Hunter (1943), which show that both glucose and lactate are oxidized vigorously by washed suspensions of *Myco. phlei*, suggest that further investi-

gation of the behaviour of these substrates might be profitable.

METHODS

Organisms and suspensions. Three strains of *Myco. phlei* were employed, no. 525 N.C.T.C. and two others apparently identical with it. The stock cultures were maintained on glycerinated Dorset's egg medium and transferred to meat infusion broth containing 5% of glycerol for several generations before use. On the 7th–9th day of incubation at 38° the bacteria were washed and suspended in distilled water by methods described previously (Edson & Hunter, 1943).

Non-viable preparations. (a) *Bacterial extracts* were prepared with the wet crushing mill of Booth & Green (1938). Bacteria (30 g.) were suspended as a creamy emulsion in 20 ml. of distilled water and crushed for 2.5 hr. About 40% of the cells were crushed, the fragments losing their acid-fast properties. The heavier debris was removed by centrifuging for 1 hr. at 4000 r.p.m. The supernatant liquid was yellowish brown and slightly opalescent.

(b) *Acetone powders.* Dry powders were made from washed bacteria by stirring and centrifuging three times with 10 vol. of cold acetone. The acetone powder was dried *in vacuo* and stored at 4°. Activity was retained for at least 5 weeks. Although acetone removes considerable amounts of pigment and lipids, the powder is yellow and does not wet readily. For manometric experiments it was converted into a uniform suspension either in distilled water or in buffer by shaking in a miniature stainless steel ball-mill (see Edson & Hunter, 1943). Suspensions usually contained 10–20 mg. of dry powder/ml.

Manometric measurements. Oxygen consumption, CO₂ production (aerobic and anaerobic) and bicarbonate were determined by the standard methods of Warburg. When HCN was used as inhibitor, the CO₂ absorbent was an appropriate mixture of KOH and KCN (Krebs, 1935). Substrates were added after equilibration. Acid substrates were used as neutral sodium salts.

Analytical methods

Determination of glucose. After a manometric experiment each cup was cooled in ice and the contents transferred to a graduated test-tube by means of a Pasteur pipette. The cup and side-bulb were washed twice with 1 ml. of distilled water, the washings being added to the test-tube in the same manner. The deproteinizing reagents (0.4 ml. 10% (w/v) sodium tungstate and 0.4 ml. 0.66N-H₂SO₄), which also flocculated the bacteria, were added together with sufficient distilled water to make a total volume of 8.0 ml. The mixture was filtered through a Whatman no. 30 paper (4.25 cm.) and a 5 ml. sample used for analysis by the method of Shaffer & Somogyi (1933).

Determination of lactic acid. The manometric cups were cooled in ice and the contents transferred (by Pasteur pipette) to a 100 ml. volumetric flask. The washings, five lots each of 1 ml., were added in the same way, followed by 4 ml. 20% (w/v) CuSO₄·5H₂O solution and 4 ml. Ca(OH)₂ suspension in succession and the volume brought to 100 ml. with distilled water. The copper-lime reagent removed the bacteria effectively. The mixture was filtered through a Whatman no. 1 paper (11 cm.) and the filtrate acidified by addition of 0.2 ml. of phosphoric acid (sp.gr. 1.7). An amount of 80 ml. was used for the determination of lactic acid by the method of Friedemann & Graesser (1933).

Detection of acetic acid. At the end of an experiment the bacterial suspension was treated with $\frac{1}{10}$ vol. of freshly prepared 7% (w/v) metaphosphoric acid and filtered through a Whatman no. 30 paper (4.25 cm.). Bacteria and dissolved proteins were thus removed. The acid filtrate was subjected to steam distillation in a micro-apparatus in which the volume (5 ml.) of the boiling liquid was kept almost constant. A volume of 20 ml. of distillate was collected, made faintly alkaline with a few drops of N-NH₄OH, and evaporated gently to 2 ml. on a hot water-bath. The concentrate was tested by the lanthanum-iodine reaction,

0.3 ml. of 5% lanthanum nitrate solution, 0.3 ml. of 0.01N-ethanolic iodine solution and 0.2 ml. of N-NH₄OH being added in succession.

RESULTS

Oxygen consumption in the presence of carbohydrates, fatty acids and related substances

The spontaneous oxygen consumption of washed suspensions of *Myc. phlei* is fairly constant for different cultures; and the oxygen uptake is augmented by several substances, notably glucose and lactate, both of which cause a five-fold increase (Table 1). Mannose, trehalose, pyruvate and *l*(-)-malate also increase the respiration. Other carbohydrates and metabolic derivatives of carbohydrate either produce no significant change, or increase oxygen consumption slightly. Of the fatty acids, formic acid is not oxidized; propionic acid causes a small rise in oxygen uptake; but acetic acid and

Table 1. *Respiration of Myco. phlei (washed suspension) in the presence of various substrates*

(Medium: 3 ml. 0.033M-phosphate buffer, pH 7.3, at 38°; gas: air; time: 2 hr.; Q_{O₂} = μ l. O₂ consumed/mg. dry wt. bacteria/hr.)

Substrate	Conc. (M)	Q _{O₂}	
		Substrate absent	Substrate present
Arabinose	0.02	-7.1	-8.1
Ribose	0.02	-5.9	-8.1
Glucose	0.02	-5.7	-25.3
Fructose	0.02	-6.5	-9.9
Fructosemonophosphate	0.01	-5.9	-8.5
Fructosediphosphate	0.01	-5.9	-8.5
Mannose	0.02	-7.7	-17.2
Galactose	0.02	-5.9	-6.7
Sucrose	0.02	-7.7	-7.6
Trehalose	0.02	-5.9	-13.2
Maltose	0.02	-5.9	-8.0
Lactose	0.02	-6.2	-6.3
Sorbitol	0.02	-6.7	-8.7
Glycerol	0.02	-5.9	-8.2
α -Glycerophosphate	0.01	-5.9	-7.2
β -Glycerophosphate	0.02	-5.9	-6.1
Gluconate	0.02	-7.5	-9.1
<i>dl</i> -Lactate	0.02	-5.9	-29.6
Pyruvate	0.02	-5.8	-11.6
Succinate	0.02	-5.7	-6.8
Fumarate	0.01	-6.2	-6.5
<i>l</i> (-)-Malate	0.02	-7.1	-11.6
Citrate	0.02	-7.7	-7.7
Formate	0.01	-6.2	-6.6
Acetate	0.02	-6.6	-11.1
Propionate	0.02	-6.1	-8.4
<i>n</i> -Butyrate	0.02	-6.1	-13.6
Crotonate	0.02	-5.9	-11.0
<i>iso</i> Valerate	0.02	-5.9	-7.7
<i>n</i> -Hexanoate	0.01	-5.9	-12.4
<i>n</i> -Octanoate	0.01	-5.9	-10.6
<i>dl</i> - β -Hydroxybutyrate	0.02	-6.7	-13.1
Acetoacetate	0.01	-8.6	-18.4

For 29 different cultures the mean Q_{O₂} in absence of added substrate was found to be -6.8 (standard deviation $\pm$ 0.9).

higher homologues possessing an even number of C atoms roughly double the respiration rate.

Suspensions which have been heated to 100° for 5 min. have no oxygen uptake either in presence or absence of substrate.

Reduction of methylene blue

Methylene blue can function as hydrogen acceptor, although it is less effective than oxygen. Washed suspensions in phosphate buffer at pH 7.4 reduce methylene blue anaerobically, and the reduction time is diminished by addition of substrates (Table 2). The rather slow reduction times stand in

Table 2. *Anaerobic reduction of methylene blue by washed suspensions of Myco. phlei*

(Each evacuated Thunberg tube contained: 1 ml. washed aqueous suspension (2 mg. wet bacteria/ml.); 2 ml. 0.1M-phosphate buffer, pH 7.4; 1 ml. methylene blue solution (1 in 5000); 0.5 ml. 0.2M-substrate solution added from hollow stopper. Temp. 38°.)

Substrate	Reduction time (min.)
Nil	220
<i>dl</i> -Lactate	110
Acetate	180
Succinate	165
<i>dl</i> - β -Hydroxybutyrate	125
Glycerol	132
Glucose	105

the same relative order as the Q_{O_2} values of Table 1. The reduction time of the blank was not lengthened by aeration for several hours at 38°.

substrates are not oxidized by a common katabolic process. HCN (0.001M) halves the 'blank' respiration and inhibits oxygen consumption in the presence of glucose by 84 %; under the same conditions respiration in the presence of lactate is inhibited by 39 % only. On the other hand, arsenite inhibits the respiration to a greater degree in the presence of lactate (35 %) than in the presence of glucose (12 %). Calculated on the basis of 'extra' oxygen uptake, the respective inhibitory effects of HCN and arsenite display an even greater contrast. This procedure, however, is dubious, because the influence of substrate on the spontaneous respiration is not easily determinable. Table 3 also shows that the oxidation of pyruvate and acetate is completely inhibited by arsenite.

Hydroxymalonate is known to inhibit the dehydrogenation of lactate by toluene-treated *Esch. coli* (Quastel & Wooldridge, 1928). Table 3 shows that *Myco. phlei* is able to oxidize hydroxymalonate. When hydroxymalonate and glucose are present together, the effect on oxygen uptake is additive; but the effect is competitive when lactate and hydroxymalonate are oxidized simultaneously, suggesting that the two α -hydroxy acids compete for the same enzyme.

Effects of crushing and of acetone treatment

The conclusion that *Myco. phlei* oxidizes glucose and lactate by essentially independent mechanisms is supported by the results of experiments on acetone powders and on extracts of crushed bacteria. Extracts were free from intact cells and coarser frag-

Table 3. *Influence of HCN, arsenite and hydroxymalonate on respiration of Myco. phlei in presence and absence of substrates*

(Washed suspension in 3 ml. 0.033M-phosphate buffer, pH 7.3, at 38°; gas: air.)

Inhibitor	Washed bacteria ≡mg. dry wt./flask	Oxygen consumption (μ l. in 1 hr.) in presence of						
		No substrate	0.01M- glucose	0.01M- <i>dl</i> -lactate	0.02M- <i>dl</i> -lactate	0.01M- pyruvate	0.02M- pyruvate	0.02M- acetate
Nil	9.6	74	279	261	—	—	—	—
HCN (0.001M)	9.6	32	44	159	—	—	—	—
Nil	10.1	61	268	234	—	114	—	—
Arsenite (0.004M)	10.1	53	238	151	—	54	—	—
Nil	9.7	46	—	—	251	—	70	67
Arsenite (0.0015M)	9.7	47	—	—	215	—	49	46
Nil	4.4	20	116	88	—	—	—	—
Hydroxymalonate (0.066M)	4.4	62	164	86	—	—	—	—

Effects of HCN, arsenite and hydroxy-malonate

Since lactate and glucose consume oxygen at approximately the same rate, it is possible that the former is an intermediate in the breakdown of the latter. This hypothesis is inconsistent with the experiments of Table 3 which show that these two

ments, but were slightly opalescent. The lactic acid oxidizing enzyme withstood both forms of treatment, whereas the systems that oxidize glucose, pyruvate and acetate were inactivated (Table 4).

There was a spontaneous oxygen uptake in the absence of added substrate with both preparations. This blank was not affected significantly by glucose

pyruvate or acetate, but in the presence of *dl*-lactate there was a rapid oxygen consumption which began to fall off within an hour. Both the bacterial extract and the suspended acetone powder failed to consume oxygen after 5 min. heating in a water-bath at 100° regardless of the addition of substrate.

Table 4. *Oxygen consumption of crushed cellular extract and of acetone powder (Myc. phlei)*

(Bacterial extract: 0.5 ml. extract + 1.5 ml. 0.1M-phosphate buffer; pH 7.3. Acetone powder: 15.6 mg. dry powder/3 ml. 0.03M-phosphate buffer; pH 7.3; gas: air; temp. 38°.)

Substrate (0.033M)	Oxygen uptake in 1 hr. (μl.)	
	Bacterial extract	Acetone powder
Nil	40	38
Glucose	41	36
<i>dl</i> -Lactate	571	367
Pyruvate	56	42
Acetate	63	34
<i>n</i> -Butyrate	53	—

Aerobic and anaerobic CO₂ production

The aerobic CO₂ production of *Myco. phlei* suspended in bicarbonate buffer solution containing glucose is equal to the oxygen uptake (Table 5). This confirms the observation of Ebina & Nakamura (1937) that there is no aerobic glycolysis. Under anaerobic conditions (Table 5) there is a small CO₂

Table 5. *Aerobic and anaerobic CO₂ production by Myco. phlei (washed suspensions)*

Aerobic

(Two-vessel method of Warburg: 2.0 ml. of fluid in the large, 6.5 ml. in the small vessel. Medium: bicarbonate saline (NaCl, 0.16M; NaHCO₃, 0.025M) in equilibrium with gas mixture of 5% CO₂ and 95% O₂; pH 7.4; washed bacteria ≡ 4.6 mg. dry wt./cup; temp. 38°; time 70 min.)

	Oxygen uptake (μl.)	CO ₂ formation (μl.)	CO ₂ /O ₂
No substrate	35.5	33.5	0.94
Glucose (0.01M)	89	90	1.01

Anaerobic

(Single conical Warburg vessels: 3 ml. bicarbonate Ringer solution (Krebs & Henseleit, 1932) in equilibrium with gas mixture of 5% CO₂ and 95% N₂. Phosphorus in the inseal; pH 7.4; washed bacteria ≡ 10.7 mg. dry wt./cup; temp. 38°; time 120 min.)

Substrate	Q _{CO₂} ^{N₂}
Nil	0.6
Glucose (0.02M)	0.7
Fructose (0.02M)	0.7
Fructose-1,6-diphosphate (0.01M)	1.0
Pyruvate (0.02M)	1.9
Acetoacetate (0.01M)	1.6

Q_{CO₂}^{N₂} = μl. CO₂/mg. dry wt. bacteria/hr.

formation in the control which is not affected significantly by the presence of glucose, fructose or hexose diphosphate. Anaerobic CO₂ formation is increased slightly in the presence of pyruvate and acetoacetate. These experiments show that there is no glycolysis under the conditions used; and that the rate of anaerobic CO₂ production from ketonic acids is not commensurate with the rate of aerobic oxidation of these substrates (Table 1).

Table 6. *Residual bicarbonate after oxidation of glucose*

(Washed suspension of *Myco. phlei* ≡ 8.1 mg. dry wt./cup; in 2 ml. bicarbonate saline (NaCl, 0.16M; NaHCO₃, 0.005M) equilibrated with gas mixture containing 2% CO₂ and 98% O₂; pH 7.2; temp. 38°; time 2 hr.)

Substrate	Bicarbonate (μl. CO ₂)	
	Initial	Final
Nil	245	250
Glucose (0.01M)	245	225

Measurement of residual bicarbonate (Table 6) reveals a diminution which may indicate the generation of small amounts of acid from glucose under aerobic conditions.

Utilization of glucose and lactate

Table 7 shows that both glucose and lactate disappear aerobically, but not anaerobically, in the presence of washed suspensions. 95% of the substrate was recovered from heated (100°) suspensions of bacteria. The deficit which occurred anaerobically is within the margin of experimental error.

The combustion of glucose and lactate is incomplete. Assuming that the endogenous respiration is unaffected by the metabolism of added substrate, the oxidation of 1 mmol. of either glucose or lactate consumes 1.3 mmol. of oxygen; but if the oxidation of substrate suppresses the endogenous respiration completely, the equivalence is 2.2 mmol. of oxygen. Although combustion is incomplete, the ratio CO₂/O₂ is approximately 1.0 with both substrates (Table 8).

Comparative rates of oxidation of glucose, lactate, pyruvate and acetate

The rates of consumption of oxygen by a washed suspension of *Myco. phlei* respiring in the presence of small amounts of the above substrates are shown in Fig. 1. The curves for pyruvate and acetate are almost linear, whereas those for glucose and lactate present an initial rapid phase before they become parallel with the pyruvate and acetate curves. At the point indicated by the arrow, glucose had disappeared from the suspension medium; addition of a further quantity of glucose beyond this point provoked a repetition of the curve.

Table 7. *Consumption of glucose and lactate by washed suspensions of Myco. phlei*

(Bacteria suspended in 3 ml. 0.033M-phosphate buffer; pH 7.3; temp. 38°.)

	Washed bacteria (≡mg. dry wt.)	Time (min.)	Substrate added	Initial amount substrate (mg.)	Final amount substrate (mg.)	Oxygen uptake (μl.)	'Extra' oxygen consumed (μl.)	Substrate dis- appearing (mg.)
Anaerobic (gas: nitrogen; phosphorus in the in- seal)	27.7	120	Nil	0.00	0.00	—	—	0.00
	27.7	120	Glucose	1.74	1.57	—	—	0.17
	34.0	120	Nil	0.00	0.00	—	—	0.00
	34.0	120	dl-Lactic acid	3.64	3.54	—	—	0.10
Aerobic (gas: air)	27.7	130	Nil	0.00	0.00	433	—	0.00
	27.7	130	Glucose	3.52	0.28	944	511	3.24
	34.0	180	Nil	0.00	0.00	361	—	0.00
	34.0	180	dl-Lactic acid	3.64	2.09	852	491	1.55

Table 8. *O₂ consumption and CO₂ production by washed suspensions of Myco. phlei*(Medium: 3 ml. 0.03M-phosphate buffer; pH 7.3 at 38°. Total CO₂ (free and bound) measured by the method of Warburg & Yabusoe (1924).)

Washed bacteria/cup (≡mg. dry wt.)	Time (min.)	Substrate	%Oxygen uptake (μl.)	Total CO ₂ (μl.)	CO ₂ /O ₂
11.0	80	Nil	86	78	0.91
11.0	80	Glucose (0.01M)	363	377	1.04
9.0	150	Nil	96	77	0.80
9.0	150	dl-Lactate (0.02M)	327	336	1.03

The data of Table 7, obtained with larger amounts of substrate, represent the initial phase of oxidation of both glucose and lactate.

Fig. 1. Oxygen consumption of a washed suspension of *Myco. phlei* (≡26 mg. dry wt. bacteria/3 ml. 0.03M-phosphate buffer, pH 7.4) at 38°. Gas: air. Initial quantity of substrate was 1/300 mmol. in each case. The arrow marks the point at which glucose had disappeared entirely from the suspension medium.

Products of oxidation

Products of incomplete combustion of glucose and lactate were sought, but in the case of glucose no product other than CO₂ has yet been identified.

When the bacteria respire in the presence of arsenite, intermediate products of lactic acid breakdown can be detected. Traces of pyruvate (indicated by a faint olive green colour in the nitroprusside test applied to a filtrate which had been deproteinized with $\frac{1}{10}$ vol. 30% (w/v) trichloroacetic acid) were found together with larger amounts of acid volatile in steam which gave the blue lanthanum-iodine reaction (Table 9).

Having regard to the aerobic conditions, the CO₂/O₂ ratio (Table 8) and the oxygen uptake/mmol. lactic acid disappearing, it is almost certain that the volatile acid which reacts with the lanthanum-iodine reagent is acetic acid. Traces of this acid were found in the absence of arsenite (Table 9).

DISCUSSION

It is widely recognized that the acid-fast bacteria do not utilize carbohydrate anaerobically. Merrill (1930, 1931 *a, b*) has also shown that cleavage products of carbohydrate metabolism do not accumulate in synthetic media on which aerobic cultures have grown.

The experiments reported in this paper show that glucose and lactic acid are not oxidized completely by 'resting cells' of *Myco. phlei*. Clifton & Logan (1939), observing that the oxidation of a number of organic compounds by suspensions of *Esch. coli* was incomplete, suggested that a portion of the substrate was assimilated in the form of carbohydrate and the remainder oxidized to CO₂ and water.

Table 9. *Products of oxidation of lactate*

(Washed suspension of *Myco. phlei*; 75 mg./ml. distilled water; temp. 38°;
gas: air; time 3.5 hr. 25 ml. stoppered conical flasks set up as follows)

Flask ...	1 (ml.)	2 (ml.)	3 (ml.)	4 (ml.)
Bacterial suspension	4.0	4.0	4.0	4.0
Phosphate buffer (0.1 M) pH 7.4	2.0	2.0	2.0	2.0
<i>dl</i> -Lactate (0.2 M)	—	1.0	—	1.0
Arsenite (0.05 M)	—	—	0.7	0.7
Distilled water	1.7	0.7	1.0	—
Lanthanum-iodine test	Neg.	±	Neg.	++++
Nitroprusside test	Neg.	Neg. or ±	Neg.	±

± = Trace.

A similar hypothesis could be applied to the glucose metabolism of *Myco. phlei* in the absence of contradictory evidence; but a different hypothesis is required to account for the incomplete combustion of lactic acid because extracellular products of intermediary metabolism have been detected.

The following sequence of aerobic reactions may be postulated:

Initial oxygen uptake is at least twice as fast in the presence of lactate as in the presence of pyruvate or acetate (Table 1 and Fig. 1). Although this rapid phase may represent the conversion of lactate to pyruvate and acetate, no more than a trace of either pyruvate or acetate can be found at any time during the oxidation.

When lactate is oxidized in the presence of arsenite (which completely inhibits the oxidation of added pyruvate or acetate (Table 3)) there is an accumulation of acetate but not of pyruvate (Table 9). Anaerobic dismutation of pyruvate, such as Krebs (1937) has described for the *Staph. aureus*, cannot account for the disappearance of pyruvate because

anaerobic CO₂ formation is much too slow (Table 5). The disappearance of pyruvate formed as an intermediate in the breakdown of lactate—as distinct from added pyruvate—could be explained by a non-enzymic coupled oxidation involving H₂O₂ and leading to the formation of acetate. This hypothesis is consistent with the accumulation of acetate in the presence of arsenite and with the relative insensitivity of lactate oxidation to HCN. It is supported by evidence obtained in a study of the system which oxidizes lactic acid in *Myco. phlei* (Edson, 1947).

Nevertheless, the hypothesis does not exclude the existence of an alternative route for the breakdown of lactic acid via pyruvic acid, since the oxidation of added pyruvate is enzymic and sensitive to arsenite. This alternative pathway may lead to processes of assimilation.

SUMMARY

1. Glucose and lactate are oxidized vigorously, but incompletely, by washed suspensions of *Myco. phlei*. They are not metabolized anaerobically.
2. Bacterial extracts and acetone powders oxidize lactate but not other substrates tested.
3. Comparative rates of oxidation of glucose, lactate, pyruvate and acetate have been determined.
4. Intermediary metabolites have been detected in the breakdown of lactate.

We are much indebted to Prof. H. A. Krebs for his kindness in reading the proofs.

REFERENCES

- Booth, V. H. & Green, D. E. (1938). *Biochem. J.* **32**, 855.
 Clifton, C. E. & Logan, W. A. (1939). *J. Bact.* **37**, 523.
 Ebina, T. & Nakamura, T. (1937). *Tohoku J. exp. Med.* **31**, 60.
 Edson, N. L. (1947). *Biochem. J.* **41**, 145.
 Edson, N. L. & Hunter, G. J. E. (1943). *Biochem. J.* **37**, 563.
 Friedemann, T. E. & Graesser, J. B. (1933). *J. biol. Chem.* **100**, 291.
 Krebs, H. A. (1935). *Biochem. J.* **29**, 1620.
 Krebs, H. A. (1937). *Biochem. J.* **31**, 661.
 Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
 Merrill, M. H. (1930). *J. Bact.* **20**, 235.
 Merrill, M. H. (1931 a). *J. Bact.* **21**, 361.
 Merrill, M. H. (1931 b). *J. Bact.* **21**, 375.
 Quastel, J. H. & Wooldridge, W. R. (1928). *Biochem. J.* **22**, 689.
 Shaffer, P. A. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 695.
 Stephenson, M. & Whetham, M. D. (1922). *Proc. Roy. Soc. B*, **93**, 262.
 Stephenson, M. & Whetham, M. D. (1924). *Proc. Roy. Soc. B*, **95**, 200.
 Warburg, O. & Yabusoe, M. (1924). *Biochem. Z.* **146**, 380.