REFERENCES

Allen, T. H. & Bodine, J. H. [1941]. *Science*, **94**, 443.
Bertrand, G. & Mallèvre, A. [1895]. *C.R. Acad. Sci., Paris*, **121**, 726.
Keilin, O. & Mann, T. [1938]. *Proc. roy. Soc.* B, **125**, 187.
—— —— [1939]. *Nature, Lond.*, **143**, 23.
—— —— [1940]. *Nature, Lond.*, **145**, 304.
Kubowitz, F. [1937]. *Biochem. Z.* **292**, 221.
—— [1938]. *Biochem. Z.* **299**, 32.
Lamb, J. & Sreerangachar, H. B. [1940]. *Biochem. J.* **34**, 1472.
Meiklejohn, G. T. & Stewart, C. P. [1941]. *Biochem. J.* **35**, 755.
Shaw, W. S. & Jones, K. B. W. [1935]. *Uni. Plant. Ass. South India Bull.* **4***b*, 23.
Shorland, F. B. & Wall, E. M. [1936]. *Biochem. J.* **30**, 1047.
Sreerangachar, H. B. [1943]. *Biochem. J.* **37**, 653, 656, 661.
Venkateswaran, S. L. & Sreenivasaya, M. [1940]. *Curr. Sci.* **9**, 21.

Oxidation of Acetic Acid in Animal Tissue

By A. KLEINZELLER, *Department of Biochemistry, University of Sheffield*

(*Received* 7 *October* 1943)

Although it is known that acetic acid is readily oxidized in the animal, little is known of the intermediate stages on its oxidative path in the animal body, and numerous schemes have been suggested for its intermediate metabolism. In the present paper the oxidation of acetic acid and related 2-carbon compounds in animal tissue was examined. The results suggest that neither glycollic acid, glyoxylic acid, nor glycine are intermediates in the oxidation of acetic acid.

In the course of the investigation it was found that glyoxylic acid is a strong inhibitor of tissue respiration and of carboxylase. This observation may be useful in the study of enzymic reactions.

METHODS

Preparation of glyoxylic acid [Mohrschulz, 1926]. The Ca-salt was isolated and recrystallized three times (analysis: Ca, found 17·9%; calc. 17·9%). The Na-salt used was prepared from the Ca-salt by an equimolecular quantity of Na_2CO_3.

Manometric experiments were carried out at 40° in Warburg's conical flasks. Fresh tissue was either sliced or minced. In every experiment tissue from the same normally fed animal was used, and the results are therefore directly comparable.

The rate of disappearance of carboxylic acids was studied by a method first described by Meyerhof & Lohmann [1926] [see also Elliott & Schroeder, 1934]. The method is based on the fact that the oxidation of a carboxylic acid in a neutral buffered medium yields, under certain conditions, bicarbonate. The increase of bicarbonate in the solution is therefore a measure of the oxidation of the organic acid. Tissue slices (dry weight 7–30 mg. according to species and tissue) were suspended in 3 ml. physiological saline [Krebs & Henseleit, 1932] containing 80 μl. bicarbonate/ml. The substrates were added as Na-salts to the main compartment of the flask. 0·2 ml. 2N NaOH was placed into the centre well and, unless otherwise stated, the gas space of the cup was filled with O_2. The manometers were put into the thermostat and the O_2 uptake was measured after an equilibration period of 10 min. The total O_2 uptake was calculated by extrapolating for the equilibration period. After the incubation period (90–120 min.) the cups were cooled with ice, and the slices removed, washed, dried, and weighed. The NaOH in the centre well was replaced by 0·1 ml. 10% H_2SO_4, and 0·2 ml. 10% H_2SO_4 was measured into the side-arm. The gas space was filled with 5% CO_2 in O_2, and, after equilibration, the amount of $NaHCO_3$ in the solutions was estimated by acidification. The values found were corrected for the amount of $NaHCO_3$ carried by the tissue slices, on the assumption that a volume of liquid equalling 10 times the dry weight of tissue is introduced or removed with the tissue. From the values obtained, Q_{O_2} and $Q_{\text{bicarbonate}}$ ($Q_{\text{bic.}}$) were calculated. $Q_{\text{bic.}}$ is equal to the negative metabolism quotient of the substrate investigated ($-Q_{\text{substrate}}$), provided that no acid groups are formed during the experiment, and the carboxylic group is oxidized. When the carboxylic group is not altered as a result of the metabolism, the changes of bicarbonate found in the experiment are not related to the utilization of the substrate.

In experiments with minced tissue, 3 ml. of a suspension of tissue in phosphate saline [Krebs, 1933] were measured into the main compartment; 0·2 ml. 2N NaOH was placed into the centre well, and the substrate was measured into the side-arm and made up to 1 ml. with the saline medium. The gas space was filled with O_2. After an equilibration period of 10 min. the contents of the cup were mixed and the O_2 uptake was measured.

In anaerobic experiments, yellow phosphorus was placed in the centre well, the gas space filled with 5% CO_2 in N_2, and the CO_2 output measured.

In some of the experiments lactic acid was estimated according to Friedemann, Cotonio & Shaffer [1927].

RESULTS

Oxidation of acetate. As measured by the formation of bicarbonate, this is more rapid in guinea-pig kidney cortex than in any other tissue examined

(see Table 1): 0·0156M acetate (final concentration) increases Q_{O_2} about 50% above that of a control without substrate, and the average $Q_{bic.}$ is +7·6 as compared with +1·0 in absence of any substrate (average of 16 experiments). Elliott & Schroeder [1934] and Elliott, Benoy & Baker [1935] found in the presence of acetate a $Q_{bic.}$ of 4·7 in rabbit and 4·1 in rat kidney. $Q_{bic.}$ in the presence of acetate increases with the concentration of substrate (Table 2).

Table 1. *Oxidation of acetate in various tissues*

Tissue	Acetate added (M)	Q_{O_2}	$Q_{bic.}$
Guinea-pig: Kidney	0	−12·5	+2·0
	0·0156	−18·1	+7·3
Liver	0	− 6·4	+0·28
	0·0156	− 7·6	+2·27
Brain	0	− 8·0	+0·56
	0·0156	− 7·5	+1·15
Rat: Kidney	0	−23·2	+1·12
	0·0156	−29·6	+4·2

Table 2. *Effect of substrate concentration on the oxidation of acetate in guinea-pig kidney cortex*

Acetate conc. (M)	Q_{O_2}	$Q_{bic.}$
0	− 9·8	+0·68
0·00125	−17·2	+3·1
0·0025	−17·3	+4·0
0·005	−18·4	+5·5
0·01	−20·0	+6·4
0·02	−21·6	+7·2

Effect of pH. The effect of pH on acetate oxidation was studied by using gas mixtures containing different concentrations of CO_2 for filling the vessels. No NaOH was placed into the centre well. A higher rate of $NaHCO_3$ formation from acetate was found when the manometers were filled with a mixture of CO_2 and O_2, instead of O_2, with NaOH in the centre well. $Q_{bic.}$ slightly decreases with increasing concentrations of CO_2 in the gas mixture (Table 3).

Table 3. *Effect of* pH *on acetate oxidation*

Gas mixture	Solution in centre well	Approx. pH (calc.)	Acetate added (M)	Q_{O_2}	$Q_{bic.}$
O_2	NaOH	7·33	0	−12·2	+1·28
O_2	NaOH	7·33	0·015	−17·7	+5·91
1% CO_2 in O_2	None	7·33	0·015	—	+7·8
5% CO_2 in O_2	None	6·6	0·015	—	+6·9
20% CO_2 in O_2	None	6·0	0·015	—	+6·32

Effect of ammonium ions. Ammonium ions have a catalytic effect on the utilization of some substrates in kidney cortex [Edson, 1935; Krebs & Cohen, 1939]. Table 4 shows that NH_4Cl inhibits the oxidation of acetate, as measured by bicarbonate formation; the O_2 uptake is also reduced. No appreciable disappearance of NH_4^+ was observed. Inhibition of respiration by NH_4Cl has been previously observed in liver [Edson, 1935], in brain [Weil-Malherbe, 1938] and in the presence of acetoacetate in kidney [Edson, 1935].

Table 4. *Effect of* NH_4Cl *on acetate oxidation*

Additions (final concn.)			
Acetate (M)	NH_4Cl (μl.)	Q_{O_2}	$Q_{bic.}$
0	—	−13·5	+1·61
0·0156	—	−22·2	+9·15
0	1120	−13·0	+0·91
0·0156	224	−16·4	+4·35
0·0156	560	−17·5	+5·78
0·0156	1120	−17·2	+3·4
0·0156	2240	−17·6	+2·92

Effect of anaerobic conditions. No bicarbonate is formed from acetate under anaerobic conditions (Table 5). This confirms unpublished experiments of Dr D. Herbert [private communication], made by different methods.

Table 5. *Effect of anaerobic conditions on the bicarbonate formation from acetate*

Acetate added (M)	CO_2 liberated (μl.)	$NaHCO_3$ found (μl.)	Total CO_2 (μl.)
0	41	235	276
0·0156	39	228	267

Effect of malonate. Malonate was found to inhibit the formation of bicarbonate from acetate in guinea-pig kidney cortex (Table 6). The inhibition is slight at a concentration of 0·001M, whilst almost complete inhibition is found at a final concentration of 0·031M. This inhibition of acetate oxidation is abolished neither by glutamate nor by glucose. Inhibition of the oxygen uptake of liver slices in the

Table 6. *Effect of malonate on the bicarbonate formation from acetate*

Exp. no.	Additions: Acetate (M)	Additions: Malonate (M)	Q_{O_2}	$Q_{bic.}$
1	—	—	−15·1	+2·18
	0·0156	—	−22·4	+9·1
	0·0156	0·031	−13·5	+1·9
2	—	—	−15·7	0·0
	—	0·01	−13·0	+0·87
	0·0156	—	−23·3	+8·52
	0·0156	0·01	−14·8	+3·7
	0·0156	0·003	−17·2	+6·4
	0·0156	0·001	−17·8	+8·0

presence of acetate has been reported by Jowett & Quastel [1935].

Oxidation of 2-carbon compounds in kidney cortex. Challenger, Subramaniam & Walker [1927] suggested that in moulds acetate is oxidized to form glycollic, glyoxylic, and oxalic acids. This scheme of oxidation has also been suggested for animal tissue [Dakin, 1922; Toeniessen & Brinkmann, 1930]. The oxidation by kidney cortex of glycollic and glyoxylic acids and of two other 2-carbon compounds, glycine and ethanol, which might possibly be expected to be connected with the metabolism of acetate, was tested (Table 7). Neither glycollate, which was shown to be oxidized by rat- and rabbit-liver preparations [Dohan, 1940], nor glycine, is appreciably oxidized by guinea-pig kidney cortex. On the other hand, glyoxylate was found to be a strong inhibitor of tissue respiration. $Q_{bic.}$ of kidney tissue in presence of 0·0156M or lower concentrations of glyoxylate was generally negative. This observation indicates the formation of free acidic groups. Ethanol has no marked effect on the O_2 uptake; this confirms the results of Leloir & Muñoz [1938].

Table 7. *Oxidation of 2-carbon compounds in guinea-pig kidney cortex*

Exp. no.	Substrate	Q_{O_2}	$Q_{bic.}$
1	—	−14·3	+0·85
	0·0156M acetate	−20·4	+6·76
	0·0156M glycollate	−14·7	+0·99
2	—	−13·0	+1·85
	0·0156M acetate	−16·6	+5·15
	0·0156M glycine	−12·6	+1·82
3	—	−13·6	0·0
	0·0156M acetate	−20·2	+5·8
	0·0156M glyoxylate	− 7·2	−3·68
	0·0065M glyoxylate	−13·5	−1·5
	0·0032M glyoxylate	−14·5	−0·44
4	—	−12·2	+1·28
	0·0156M acetate	−17·7	+5·91
	0·0156M ethanol	−12·55	+1·12

Inhibition by glyoxylate. Glyoxylate has a strong inhibitory effect on the respiration of liver, muscle and kidney. In minced pigeon-breast muscle and liver the inhibition of respiration increases with time (Fig. 1; Table 8). The glyoxylate inhibition in pigeon-breast muscle is not abolished by citrate, fumarate or pyruvate, and only slightly by α-keto-glutarate or succinate.

Table 8. *Effect of glyoxylate on the respiration of minced pigeon-breast muscle and liver*

Cup	Tissue	Tissue wet wt. (mg.)	Glyoxylate (M)	O_2 uptake/ 60 min. (μl.)
1	Muscle	250	0	1179
2	Muscle	250	0·00625	441
3	Liver	400	0	865
4	Liver	400	0·00625	443

Under anaerobic conditions, acid or CO_2 production of guinea-pig kidney cortex and of liver is increased by the presence of glyoxylate. No increased formation of lactic acid in the presence of glyoxylate was observed in liver tissue (Table 9). Glyoxylate therefore does not increase the glycolysis of liver, and it may be assumed that the formation of free acidic groups in the presence of glyoxylate under anaerobic conditions is due to a coupled oxido-reduction of the substrate according to the reaction:

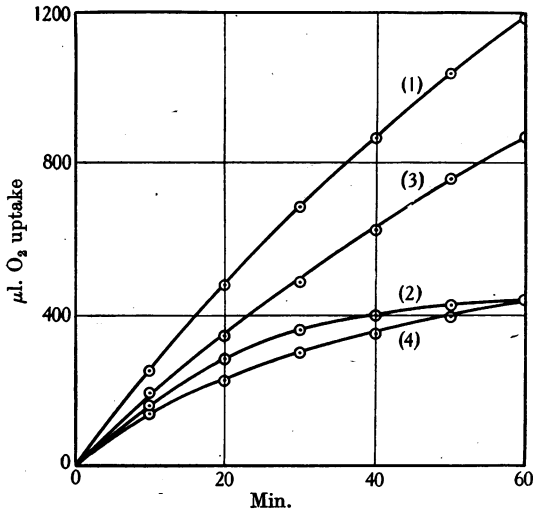

Fig. 1. The effect of 0·00625M glyoxylate on the respiration of minced pigeon-breast muscle and liver. The numbers of the curves correspond with the numbers of cups in Table 8.

$$2\mathrm{COOH.CHO} + \mathrm{H_2O} = \mathrm{COOH.COOH} + \mathrm{COOH.CH_2OH}.$$

Dismutation of glyoxylic acid according to the above reaction has been reported to occur in the presence of strong alkali [Böttinger, 1880], or of yeast maceration juice [Stepanow & Kusin, 1930].

Table 9. *Effect of glyoxylate on formation of* CO_2 *and lactic acid under anaerobic conditions*

No.	Tissue (of guinea-pig)	Glyoxylate (M)	Q_{CO_2}	Q_{lactic}
1	Kidney	0	1·39	—
		0·0143	3·13	—
	Liver	0	1·85	—
		0·0143	3·0	—
2	Liver	0	3·56	3·87
		0·0143	5·30	3·83

Effect of glyoxylate on carboxylase. The decarboxylation of pyruvate by fresh yeast maceration juice is inhibited by glyoxylate. This inhibition

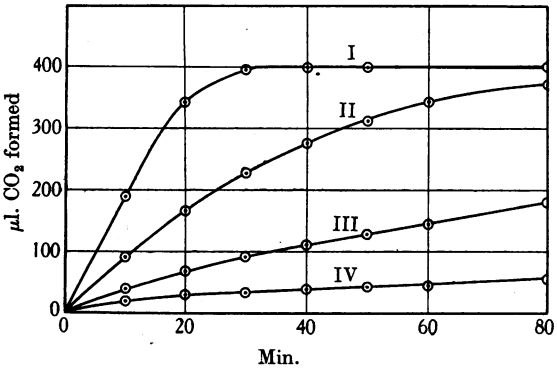

Fig. 2. Effect of glyoxylate on the decarboxylation of pyruvate by yeast maceration juice. Cup content: 400µl. pyruvate, 0·3 ml. 3M acetate buffer pH 5·0, 3·5 ml. H_2O. Side-arm: 1 ml. yeast maceration juice. Volume made to 5 ml., temp. 25°. Conc. of glyoxylate: I = 0; II = 0·001M; III = 0·002M; IV = 0·004M.

increases with increasing concentrations of glyoxylate (Fig. 2). It is probable that glyoxylate competes with pyruvate for the active groups of carboxylase.

DISCUSSION

The investigation of the oxidation of glycine, glycollic and glyoxylic acids in guinea-pig kidney cortex, as measured by the formation of bicarbonate, has shown that none of these substrates can be an intermediate in the oxidative path of acetate. Apart from the substrates investigated, the only substance that could arise by direct oxidation of acetic acid is oxalic acid, which is known to be little utilized in animal tissue [Dakin, 1922]. It seems that the oxidation of acetic acid follows other pathways, probably involving condensation reactions. Condensations of acetic acid have been demonstrated by Loeb [1912], who found that acetoacetic acid is formed from acetic acid, and recently Slade & Werkman [1942], using isotopic carbon, demonstrated the condensation of acetic acid to succinic acid and butylene glycol in bacteria. Lipmann [1941] suggests that acetate condenses with phosphate to form acetylphosphate before undergoing oxidation.

SUMMARY

1. The oxidation of acetate, glycollate, and glyoxylate in guinea-pig kidney cortex was studied by examining the effect of these substrates on the O_2 uptake and the formation of bicarbonate.

2. The oxidation of acetate, as measured by the formation of bicarbonate, was more rapid in guinea-pig kidney cortex than in any other tissue examined. On the average, 0·0156M acetate raised the Q_{O_2} by about 50% and $Q_{bic.}$ was +7·6 as compared with a $Q_{bic.}$ + 1·0 in absence of added substrate.

3. The oxidation of acetate was found to increase with the substrate concentration; it depends on the pH, the highest $Q_{bic.}$ having been found when the pH of the medium was decreased by filling the vessel with 1% CO_2 in O_2; NH_4^+ decreased the oxidation of acetate; no bicarbonate was formed under anaerobic conditions.

4. Malonate inhibited the oxidation of acetate in guinea-pig kidney. The inhibition was almost complete at a concentration of 0·031M malonate.

5. Glycine, glycollic acid, and ethanol were not appreciably oxidized in guinea-pig kidney. Glyoxylic acid is a strong inhibitor of tissue respiration.

6. Glyoxylic acid strongly inhibits the decarboxylation of pyruvate by fresh yeast maceration juice.

7. Glyoxylic acid to a small extent may undergo a coupled oxido-reduction in liver and kidney tissue to form glycollic and oxalic acids.

8. It is concluded that neither glycollic nor glyoxylic acid is an intermediate in the oxidation of acetic acid in guinea-pig kidney cortex.

The author wishes to express his thanks to Dr H. A. Krebs for having suggested this investigation, and for his constant help and advice.

REFERENCES

Böttinger, C. [1880]. *Ber. dtsch. chem. Ges.* **13**, 1932.
Challenger, F., Subramaniam, V. & Walker, T. K. [1927]. *J. chem. Soc.* p. 200.
Dakin, H. D. [1922]. *Oxidations and Reductions in the Animal Body*, 2nd ed. London: Longmans, Green.
Dohan, J. S. [1940]. *J. biol. Chem.* **135**, 793.
Edson, N. L. [1935]. *Biochem. J.* **29**, 2082.
Elliott, K. A. C., Benoy, M. P. & Baker, Z. [1935]. *Biochem. J.* **29**, 1937.
—— & Schroeder, E. F. [1934]. *Biochem. J.* **28**, 1920.
Friedemann, T. E., Cotonio, M. & Shaffer, P. A. [1927]. *J. biol. Chem.* **73**, 359.
Jowett, M. & Quastel, J. H. [1935]. *Biochem. J.* **29**, 2181.
Krebs, H. A. [1933]. *Hoppe-Seyl. Z.* **217**, 191.
Krebs, H. A. & Cohen, P. P. [1939]. *Biochem. J.* **33**, 1895.
—— & Henseleit, K. [1932]. *Hoppe-Seyl. Z.* **210**, 33.
Leloir, L. F. & Muñoz, J. M. [1938]. *Biochem. J.* **32**, 299.
Lipmann, F. [1941]. *Advances of Enzymology*, **1**, 99.
Loeb, A. [1912]. *Biochem. Z.* **47**, 118.
Meyerhof, O. & Lohmann, K. [1926]. *Biochem. Z.* **171**, 381.
Mohrschulz, W. [1926]. *Z. Elektrochem.* **32**, 434.
Slade, H. D. & Werkman, C. H. [1942]. *Arch. Biochem.* **2**, 97.
Stepanow, A. & Kusin, A. [1930]. *Ber. dtsch. chem. Ges.* **63**, 1147.
Toeniessen, E. & Brinkmann, E. [1930]. *Hoppe-Seyl. Z.* **187**, 137.
Weil-Malherbe, H. [1938]. *Biochem. J.* **32**, 2257.