SUMMARY

1. The rate of the condensation of glyoxylate and oxaloacetate was followed by determining the disappearance of the aldehyde group.

2. The reaction was complete after 3 hr. at 40° and pH 7·4, and did not occur at pH 3. The compound obtained was identified as the trihydrate of the trisodium salt of α-hydroxy-β-oxalosuccinic acid (oxalomalic acid).

3. A similar compound, but C_5 instead of C_6, namely α-hydroxymethyl-β-oxosuccinic acid, was obtained when formaldehyde was substituted for glyoxylate in the condensation.

4. Only oxalomalate was able to inhibit the activity of crude or purified preparations of aconitase. The inhibitory effect was of the competitive type, with either *cis*-aconitate or citrate as substrate.

5. A possible physiological role of glyoxylate to control the rate of the citric acid cycle, through the condensation with oxaloacetate, is suggested.

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Control of the Citric Acid Cycle by Glyoxylate

2. MECHANISM OF THE INHIBITION OF RESPIRATION IN LIVER AND KIDNEY PARTICLES

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The observation that the chemical reaction of glyoxylate with oxaloacetate produced the formation of oxalomalate, a new tricarboxylic acid, which inhibits the activity of crude and purified aconitase (Ruffo, Romano & Adinolfi, 1959; Ruffo, Testa, Adinolfi & Pelizza, 1962), led us to investigate the possible physiological significance of this reaction in animal tissues. Since both glyoxylate and oxaloacetate are cellular metabolites, it seems possible that glyoxylate might control the citric acid cycle, owing to its property of reacting with oxaloacetate to form a powerful inhibitor of aconitase. To investigate this problem, we determined the oxygen uptake, the P:O ratio and the formation of citrate in intact or damaged rat-liver mitochondria and in rabbit-kidney cyclophorase preparations, incubated with different substrates of the citric acid cycle in the presence and in the absence either of glyoxylate (with and without oxaloacetate) or of oxalomalate.

The main results obtained showed that glyoxylate, alone or with oxaloacetate, and oxalomalate, always produced inhibition of the respiratory processes, but without an equivalent depression of the P:O ratio. The inhibition, in many cases, was accompanied by an accumulation of citrate.

EXPERIMENTAL

Preparation and incubation of intact and damaged mitochondria. The livers of adult albino rats were homogenized in 0·25 M-sucrose with a Potter glass homogenizer and centrifuged for 10 min. at 600*g* to sediment nuclei and cell debris, according to Schneider (1949). The supernatant was centrifuged for 15 min. at 8500*g*, and the sedimented mitochondria were washed twice with 0·25 M-sucrose and resuspended in the same solution. Samples

corresponding to about 1 mg. of N were pipetted into one of two different media contained in Warburg vessels. Medium *A* was modified from that of Judah (1951), and contained, in 2 ml.: substrates, 16 μmoles; oxaloacetate, 4 μmoles; KCl, 45 μmoles; Na_2HPO_4 (adjusted to pH 7·4 with N-HCl), 40 μmoles; ATP, 2 μmoles; $MgSO_4$, 10 μmoles; cytochrome *c*, 0·022 μmole; glucose, 30 μmoles; added last was hexokinase (Sigma III type), 100 K.M. units (Kunitz & McDonald, 1948). Glyoxylate, as indicated, and 0·6 ml. of the mitochondrial suspension, were added. Medium *B* was that of Lardy & Wellman (1952), with the following composition, in 2 ml.: substrates, 21 μmoles; ATP, 3·5 μmoles; KH_2PO_4 (adjusted to pH 7·4 with anhydrous K_2HPO_4), 30 μmoles; cytochrome *c*, 0·022 μmole; glucose, 35 μmoles; hexokinase (Sigma III type), 100 K.M. units (in the side arm). Oxaloacetate, oxalomalate and glyoxylate, as indicated, and 0·7 ml. of mitochondria suspended in 0·25 M-sucrose, were added. After equilibration for 5 min., the incubation was started by tipping the contents of the side arm into the main chamber, and the O_2 uptake was measured with the conventional Warburg apparatus. Incubation was carried out for 25 min. in air at 30°, unless otherwise stated.

For uncoupling phosphorylation in mitochondria, either 2,4-dinitrophenol (final concn. 30 μM) was tipped in from the side arm, instead of hexokinase, into medium *A*, or the incubation was carried out in an acidic hypo-osmotic medium. For this last experiment mitochondria were prepared by homogenizing and centrifuging the livers in 0·25 M-sucrose, 1 mM with respect to EDTA (disodium salt), not neutralized (see Table 1). The sediment obtained at 8500*g* was collected, resuspended in the same solution, and 3 ml. of this suspension (corresponding to about 3 mg. of mitochondrial N) was pipetted into Warburg vessels containing, in 1 ml.: 8 μmoles of Na_2HPO_4, 8 μmoles of $MgCl_2$, 8 μmoles of pyruvate or oxaloacetate or both, and 2 μmoles of glyoxylate, when present. The pH of this solution was about 6·2, and the time of incubation 1 hr. at 37°.

Preparation and incubation of cyclophorase. Rabbit-kidney cortex was extracted with 0·9% KCl solution according to Green, Loomis & Auerbach (1948) and centrifuged at 2000*g* for 15 min. The washed precipitate was suspended in the same solution, and 1 ml., containing about 50 mg. of kidney (dry wt.), was added to the incubation mixtures, containing, in a final volume of 2 ml.: 10 μmoles of Na_2HPO_4 (adjusted to pH 7·4 with N-HCl); 10 μmoles of $MgCl_2$; substrates and glyoxylate as indicated. Incubation was in air at 37°. The pH at the end of the incubation had risen to 7·6–7·8. 2,4-Dinitrophenol, at a concentration of 80 μM, was used as an uncoupling agent.

Chemical determinations. The samples were deproteinized by centrifuging after the addition either of 1 ml. of freshly prepared 5% (w/v) tungstic acid, when citrate was to be determined according to the method of Ettinger, Goldbaum & Smith (1952), or of 0·5 ml. of 20% (w/v) trichloroacetic acid, when inorganic phosphates were to be determined according to the method of Fiske & Subbarow (1925).

The substrates, coenzymes and other reagents were products of Sigma Chemical Co. and Eastman Kodak Co. (U.S.A.), L. Light and Co. Ltd., British Drug Houses Ltd. and Carlo Erba (Italy).

RESULTS

Inhibition of oxygen uptake in intact mitochondria. These experiments were carried out by incubating mitochondria with L-glutamate, oxoglutarate or pyruvate, each with a small amount of oxaloacetate suspended in medium *A* in the presence and in the absence of glyoxylate. The results (Fig. 1) showed that, whichever substrate was oxidized, glyoxylate inhibited oxygen uptake without a parallel decrease of the P:O ratio.

Inhibition of oxygen uptake after uncoupling of the phosphorylative reactions. Since the above results showed that glyoxylate plus oxaloacetate produced about the same inhibitory effect on the consumption of either oxygen or phosphates, we supposed that the depression of phosphorylation was

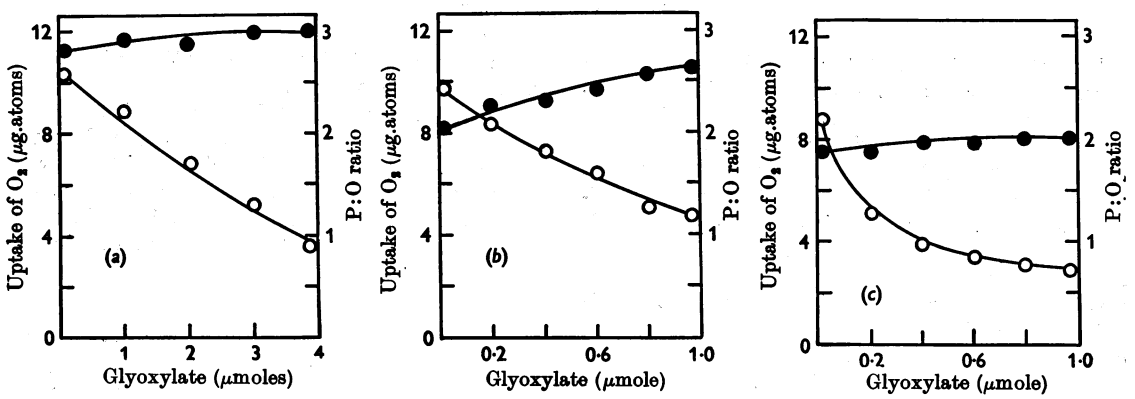

Fig. 1. Inhibition of respiration by glyoxylate and oxaloacetate in intact mitochondria. The Warburg vessels contained, in 2 ml. of medium *A*: 0·6 ml. of mitochondria suspended in 0·25 M-sucrose (corresponding to about 1 mg. of N); substrates, 16 μmoles; oxaloacetate, 4 μmoles; glyoxylate, as indicated. Incubation was in air for 25 min. at 30°. Deproteinization was carried out with 0·5 ml. of 20% (w/v) trichloroacetic acid. Substrates: (*a*) L-glutamate; (*b*) oxoglutarate; (*c*) pyruvate. ○, Uptake of O_2 (μg.atoms); ●, P:O ratio.

Table 1. *Inhibition of respiration and the accumulation of citrate in damaged mitochondria*

Mitochondria were prepared and suspended in 0·25 M-sucrose, 1 mM with respect to EDTA (disodium salt), not neutralized. A sample (3 ml.) of the suspension (corresponding to about 3 mg. of N) was added to 1 ml. of a medium containing 8 μmoles of Na_2HPO_4 and 8 μmoles of $MgCl_2$. The pH was about 6·2. Substrates (8 μmoles) and glyoxylate (2 μmoles) were added as indicated. Incubation was for 1 hr. at 37°. Deproteinization was carried out with 3 ml. of 5% (w/v) tungstic acid.

Additions	Uptake of O_2 (μl.)	Inhibition (%)	Citrate found (μmoles)	Extra citrate accumulated (μmoles)
Pyruvate	240	—	0·5	—
Pyruvate + glyoxylate	125	52	0·6	0·1
Oxaloacetate	450	—	0·2	—
Oxaloacetate + glyoxylate	120	72	2·5	2·3
Pyruvate + oxaloacetate	880	—	0·4	—
Pyruvate + oxaloacetate + glyoxylate	280	68	5·4	5·0

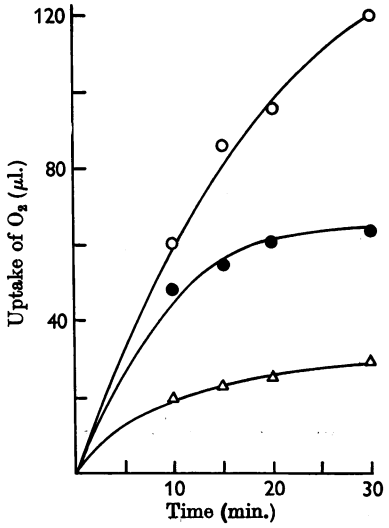

Fig. 2. Inhibition of respiration in intact mitochondria after the uncoupling of phosphorylation with 2,4-dinitrophenol. Experimental conditions were the same as in Fig. 1. Substrates were L-glutamate (16 μmoles) and oxaloacetate (4 μmoles). ○, No further additions; ●, glyoxylate (4 μmoles) added; △, glyoxylate (4 μmoles) and 2,4-dinitrophenol (0·06 μmole) added, and hexokinase omitted.

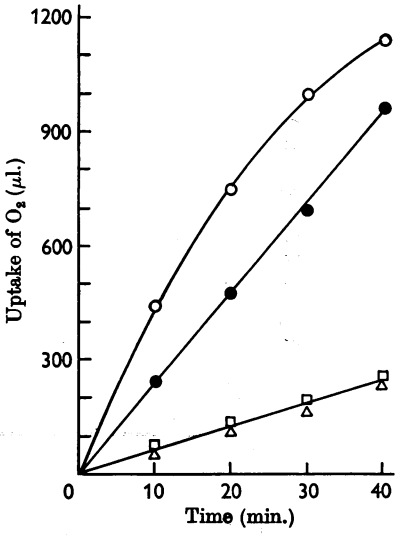

Fig. 3. Inhibition of respiration in cyclophorase preparations. The Warburg vessels contained, in a final volume of 2 ml.: 1 ml. of tissue suspension (corresponding to 50 mg. dry wt. of kidney) in 0·9% KCl; pyruvate, 16 μmoles; oxaloacetate, 8 μmoles; 2,4-dinitrophenol, when present, 0·16 μmole; glyoxylate, when present, 4 μmoles. Incubation was in air at 37°. ●, Pyruvate plus oxaloacetate; ○, pyruvate plus oxaloacetate and 2,4-dinitrophenol; △, pyruvate plus oxaloacetate and glyoxylate; □, pyruvate plus oxaloacetate, glyoxylate and 2,4-dinitrophenol.

caused by the decreased rate of respiration. If this supposition were correct the oxygen uptake would be depressed to the same extent after the uncoupling of the phosphorylative reactions. Therefore mitochondria were incubated with L-glutamate in medium *A* containing oxaloacetate and glyoxylate, in the presence and in the absence of 2,4-dinitrophenol.

The results (Fig. 2) showed that the inhibition of the respiration produced by the addition of glyoxylate to the incubation mixture containing glutamate and oxaloacetate increased in the presence of 2,4-dinitrophenol. Those reported in Table 1 confirmed that glyoxylate depressed oxygen uptake, even in damaged mitochondria. At the end of the incubation an accumulation of citrate was found in the samples with glyoxylate and oxaloacetate.

Inhibition of oxygen uptake in cyclophorase preparations. Similar results were obtained by incubating cyclophorase, prepared from rabbit kidney, with pyruvate or oxaloacetate or both in the presence and in the absence of glyoxylate and 2,4-dinitrophenol. The results (Fig. 3) showed that glyoxylate produced the same inhibition of the oxygen uptake either in the presence or in the

absence of 2,4-dinitrophenol. From these results it appears that the inhibition of oxygen uptake produced by glyoxylate plus oxaloacetate was independent of phosphorylation.

Inhibition of the oxidation of pyruvate and of citrate. To establish whether the accumulation of citrate produced by glyoxylate plus oxaloacetate was due to the inhibition of the respiration and dependent on the nature of the substrate oxidized, experiments were carried out with pyruvate (with fumarate as primer) and with citrate as substrates. For comparison, the effect of glyoxylate alone was determined in parallel experiments in which oxaloacetate was omitted.

The results (Figs. 4*a* and 4*b*) showed that even glyoxylate alone depressed the oxygen uptake in mitochondria incubated with pyruvate, and citrate accumulated. These effects were proportional to the concentration of glyoxylate present, and the addition of oxaloacetate increased both the inhibition of oxygen uptake and the accumulation of citrate. The experiments with citrate as the substrate (Figs. 4*c* and 4*d*) confirmed the results with pyruvate, since higher degrees of inhibition were obtained when glyoxylate and oxaloacetate were added together. Inhibition of citrate oxidation was shown with a concentration of glyoxylate about one-tenth of that necessary to produce in-

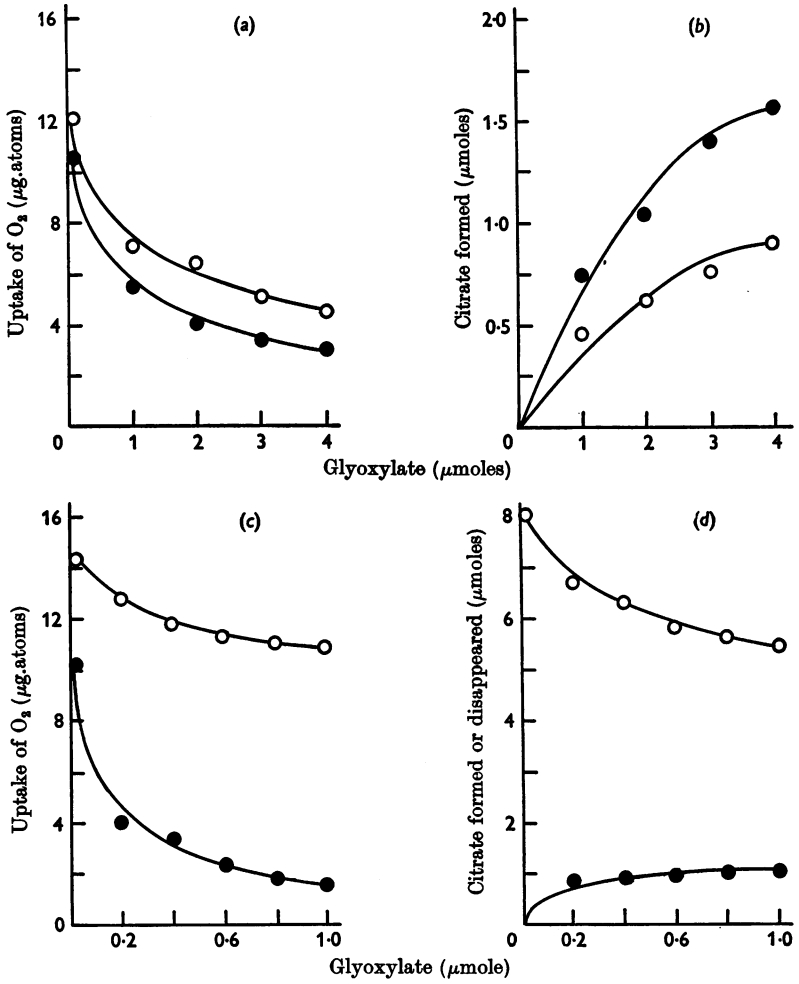

Fig. 4. Inhibition of respiration and formation of citrate in mitochondria incubated with pyruvate or citrate. ○, Incubation mixtures contained 21 μmoles of substrates and mitochondria (corresponding to about 1 mg. of N) in 2 ml. of medium *B*; ●, incubation mixtures contained in addition 4 μmoles of oxaloacetate. Glyoxylate was added as indicated. Incubation was for 25 min. at 30°. Deproteinization was carried out with 1 ml. of 5% (w/v) tungstic acid. (*a*) Uptake of O_2 and (*b*) citrate accumulation with pyruvate as substrate and 4 μmoles of fumarate as primer; (*c*) uptake of O_2 and (*d*) citrate accumulation or disappearance with citrate as substrate.

hibition with pyruvate as substrate. The disappearance of citrate, in the experiments in which glyoxylate alone was added, decreased with increasing concentrations of glyoxylate, whereas the further addition of oxaloacetate produced an extra synthesis of citrate. The inhibitory effect produced by glyoxylate alone on the oxidation either of pyruvate or of citrate may be explained by supposing that glyoxylate reacted with oxaloacetate originally present in the liver mitochondria (Kalnitsky & Tapley, 1958), and with that formed from the oxidative transformations of the substrates.

Inhibition of the oxidation of oxoglutarate and of succinate. Since the main oxidative transformations of oxoglutarate and succinate in mitochondria are several reactions from the step controlled by aconitase, it is expected that the oxidation of these substances either would not be affected or would be less inhibited by glyoxylate and oxaloacetate. Since preliminary experiments showed that, on the contrary, glyoxylate produced the same inhibition as observed when pyruvate or citrate was the substrate, we supposed that another mechanism of inhibition may be involved.

Therefore we incubated oxoglutarate and succinate with various concentrations of glyoxylate with or without oxaloacetate, and determined citrate at the end of the incubation. The results (Fig. 5) showed, with both the substrates, an

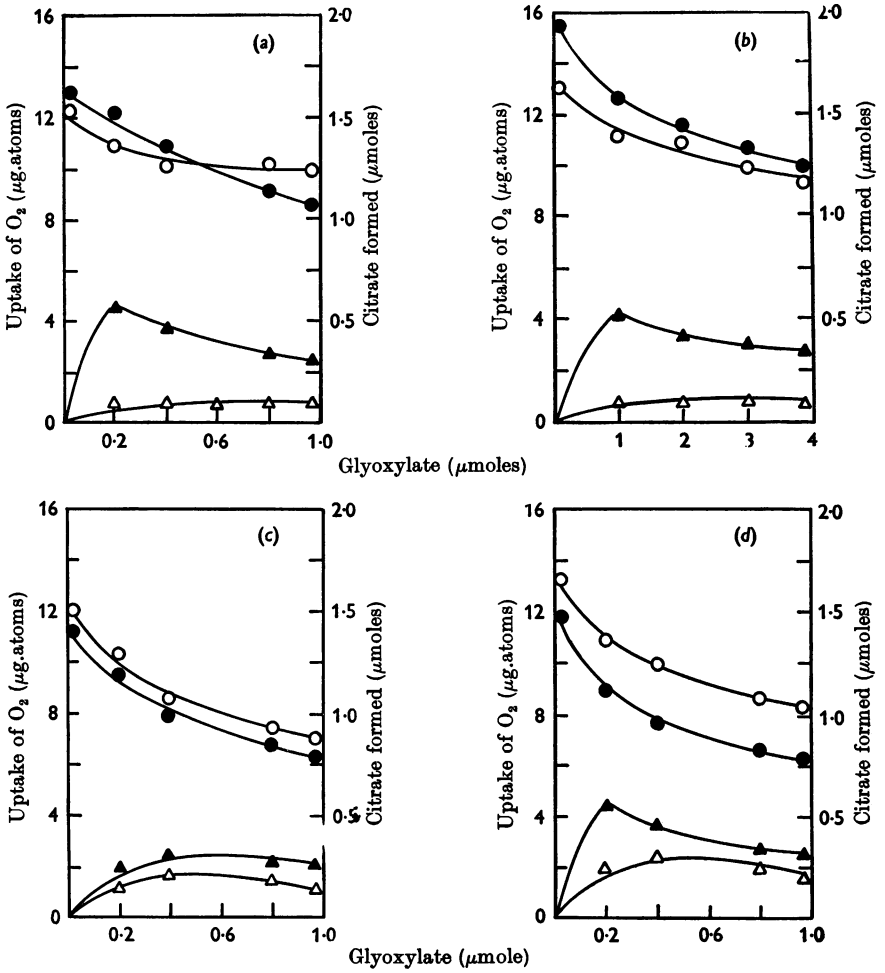

Fig. 5. Inhibition of respiration and formation of citrate in mitochondria incubated with oxoglutarate or succinate. Experimental conditions were the same as in Fig. 4. Glyoxylate was added as indicated. Substrates: (*a*) and (*b*) oxoglutarate; (*c*) and (*d*) succinate. ○, Uptake of O_2; △, citrate accumulated at the end of incubation. ● and ▲, Incubation mixtures contained in addition 4 μmoles (*a* and *b*), 1 μmole (*c*) or 2 μmoles (*d*) of oxaloacetate.

appreciable inhibition of oxygen uptake by glyoxylate only, even at very low concentrations, but the accumulation of citrate was not found. The addition of oxaloacetate to glyoxylate, with oxoglutarate as the substrate (Fig. 5*a*), either increased slightly the inhibition of oxygen uptake when the concentration of glyoxylate was low, or did not modify the inhibition when it was higher (Fig. 5*b*). Also, with succinate as the substrate (Figs. 5*c* and 5*d*), the addition of oxaloacetate produced a slight increase in the inhibition. In these experiments (Figs. 5*c* and 5*d*) less oxaloacetate was added, because this substance inhibits succinate dehydrogenase (Singer, Kearney & Massey, 1957; Gaull & Villee, 1960; Tyler, 1960). However, the concentration used (0·5–1·0 μmole/ml.) was enough to give rise to the inhibitor of aconitase during the period of incubation (see Ruffo *et al.* 1959). Accordingly a slightly increased accumulation of citrate was found with both the substrates when oxaloacetate was added, but was lower with increasing concentrations of glyoxylate. By comparing these results with those above, it appears that, whereas the inhibition of the oxygen uptake produced by glyoxylate only on succinate and oxoglutarate was almost the same as that on pyruvate and citrate, the accumulation of citrate was not the same. Even after the addition of oxaloacetate, the formation of citrate increased only slightly, and was not proportional to the concentration of glyoxylate.

Comparison between the inhibition by glyoxylate and by oxalomalate. The different behaviour of glyoxylate in inhibiting the oxidation of the different substrates tested may be explained on the basis of several different mechanisms of inhibition of the enzymes of the cycle. Since oxalomalate has been demonstrated (Ruffo *et al.* 1962) to act on aconitase, in the following experiments we compared its effect with that of glyoxylate. The effect of equimolecular concentrations of these two inhibitors on mitochondria with pyruvate plus fumarate, citrate, oxoglutarate or succinate as substrates was determined. At the end of the incubation citrate was determined.

The results (Table 2) showed that, when the substrates were pyruvate or citrate, the highest inhibition was produced by oxalomalate, but that, when the substrates were oxoglutarate or succinate, glyoxylate produced an inhibition of oxygen uptake about 50 % greater than that produced by oxalomalate. The accumulation of citrate was about the same during the incubation of citrate or pyruvate, but with oxoglutarate and succinate no accumulation of citrate was caused by glyoxylate, though a small amount was caused by oxalomalate.

Partial protection of the inhibition by pyruvate. The different mechanism of inhibition presented by glyoxylate on the oxidation of oxoglutarate and of succinate confirmed that glyoxylate might interfere also with some other enzyme of the cycle besides aconitase. Because of the molecular similarity between glyoxylate and acetate, and the diminution of citrate synthesis found on increasing the concentration of glyoxylate (see Fig. 5), glyoxylate might compete with acetate and interrupt the cycle with consequent accumulation of oxaloacetate formed from either succinate or oxoglutarate. If this were the case an excess of 'active' C_2 compounds, restoring the oxidation of oxaloacetate, would counteract the inhibition. Therefore experiments were carried out in which oxoglutarate and succinate were incubated with glyoxylate and pyruvate, added in equimolecular concentrations or in small excess. Similar experiments were done

Table 2. *Comparison between the inhibition produced by glyoxylate and by oxalomalate*

Rat-liver mitochondria (corresponding to about 1 mg. of N) were incubated for 30 min. at 30° in 2 ml. of medium *B*. Citrate (10 μmoles), other substrates (21 μmoles) and inhibitors (3 μmoles) were added as indicated. Deproteinization was carried out with 1 ml. of 5 % (w/v) tungstic acid.

Expt. no.	Additions	Uptake of O_2 (μg.atoms)	Inhibition (%)	Citrate found (μmoles)	Extra citrate accumulated (μmoles)
1	Pyruvate + fumarate	15·8	—	1·91	—
	Pyruvate + fumarate + glyoxylate	10·6	33	5·65	3·74
	Pyruvate + fumarate + oxalomalate	5·9	62	6·00	4·09
2	Citrate	12·9	—	5·90	—
	Citrate + glyoxylate	5·6	57	9·50	3·60
	Citrate + oxalomalate	3·0	77	11·72	5·82
3	Oxoglutarate	13·0	—	0·43	—
	Oxoglutarate + glyoxylate	8·5	34	0·55	0·12
	Oxoglutarate + oxalomalate	11·5	12	1·25	0·82
4	Succinate	13·6	—	0·52	—
	Succinate + glyoxylate	7·3	47	0·57	0·05
	Succinate + oxalomalate	9·8	28	1·69	1·17

Table 3. *Effect of addition of pyruvate on the inhibition by glyoxylate*

Rat-liver mitochondria (corresponding to about 1 mg. of N) were incubated for 15 and 30 min. at 30° in 2 ml. of medium *B*. The substrates added were: Expt. 1, oxoglutarate (21 μmoles); Expt. 2, succinate (21 μmoles); Expt. 3, citrate (10 μmoles). Glyoxylate (3 μmoles in Expt. 1; 2 μmoles in Expts. 2 and 3) and pyruvate (3 μmoles) were added as indicated. Deproteinization was carried out with 1 ml. of 5% (w/v) tungstic acid.

		After 15 min.			After 30 min.		
Expt. no.	Additions	Uptake of O_2 (μg.atoms)	Inhibition (%)	Citrate found (μmoles)	Uptake of O_2 (μg.atoms)	Inhibition (%)	Citrate found (μmoles)
1	None	7·9	—	—	14·8	—	0·4
	Glyoxylate	6·6	17	—	12·0	20	0·4
	Pyruvate	7·8	—	—	15·5	—	1·1
	Glyoxylate + pyruvate	8·2	0	—	14·1	9	1·6
	Pyruvate	7·8	—	1·1	—	—	—
	Glyoxylate + pyruvate	7·6	3	1·2	—	—	—
2	None	8·3	—	—	15·0	—	0·4
	Glyoxylate	6·1	25	—	8·3	45	0·4
	Pyruvate	8·1	—	—	16·5	—	0·8
	Glyoxylate + pyruvate	8·6	0	—	13·2	20	1·5
	Pyruvate	8·4	—	1·0	—	—	—
	Glyoxylate + pyruvate	8·3	0	1·2	—	—	—
3	None	6·7	—	—	14·2	—	5·3
	Glyoxylate	3·7	45	—	6·0	58	7·6
	Pyruvate	7·5	—	—	15·9	—	7·3
	Glyoxylate + pyruvate	3·9	48	—	5·1	68	10·1
	Pyruvate	7·1	—	7·9	—	—	—
	Glyoxylate + pyruvate	3·5	50	9·0	—	—	—

also with citrate as substrate. The results of these experiments (Table 3) showed that with oxoglutarate and succinate the addition of pyruvate abolished the inhibition produced by glyoxylate completely after 15 min., and partially after 30 min. No citrate accumulation was found during the first 15 min. of incubation, and there was only a slight increase during the second 15 min. of incubation. On the other hand, when citrate was the substrate, no protection was found either after 15 or after 30 min. of incubation, and the disappearance of citrate was strongly inhibited, as shown (Figs. 4*b* and 4*c*). Further, it appears that the presence of pyruvate increased the inhibition of citrate oxidation.

DISCUSSION

The results showing that glyoxylate and oxaloacetate depressed the oxygen uptake, but not the P:O ratio, suggested that the inhibition may be caused by oxalomalate, the newly discovered tricarboxylic acid formed by the chemical reaction of glyoxylate with oxaloacetate. Since oxalomalate has been isolated and identified as a competitive inhibitor of aconitase (Ruffo *et al.* 1962), its effect on this enzyme might well explain the depression of the respiration observed in these experiments, and also after the uncoupling of oxidative phosphorylation. In agreement with this possibility, the addition of oxalomalate itself to mitochondria also inhibited the rate of the respiration of all the substrates investigated.

The experiments in which glyoxylate was added without oxaloacetate gave results that indicated other possibilities. In fact, glyoxylate produced different effects with citrate and pyruvate on the one hand and with succinate and oxoglutarate on the other. In the first case the accumulation of citrate found at the end of the incubation confirmed that the inhibition may be produced by the effect on aconitase. But when oxoglutarate or succinate was the substrate, only very little citrate accumulated, and this was diminished by increasing the concentration of glyoxylate. Since the oxidation of oxoglutarate and succinate by respiring mitochondria may not be under the direct control of aconitase, this enzyme may not be responsible for the depression of the respiration; therefore an interference by glyoxylate with another reaction of the cycle may be supposed. In agreement with this possibility, glyoxylate and oxalomalate inhibited the oxygen uptake with various substrates of the cycle to different extents. In addition to an effect of oxalomalate on aconitase, glyoxylate may produce its effect on the enzymes responsible for citrate synthesis. The addition of small amounts of pyruvate, which will be transformed into acetyl-CoA, abolished the inhibition by glyoxylate of the oxidation of oxoglutarate and succinate. On the other hand, when

citrate was the substrate, no protection by pyruvate was found; in this case the enzymes related to citrate synthesis were not involved, and the inhibition would depend only on the block of aconitase.

These results indicate that glyoxylate acts in two different ways, both connected with the control of the rate of the citric acid cycle. The first involves the inhibition of aconitase by oxalomalate produced by condensation with oxaloacetate; the second may be a possible competition with acetate at the level of the enzyme system related to citrate synthesis.

SUMMARY

1. The effect of the addition of small amounts of glyoxylate (with or without oxaloacetate), and of oxalomalate, to respiring rat-liver mitochondria and to rabbit-kidney cyclophorase preparations was investigated under different conditions.

2. Glyoxylate plus oxaloacetate strongly inhibited the oxidation of L-glutamate, oxoglutarate or pyruvate in intact mitochondria, without a parallel decrease of the P:O ratio.

3. When oxidative phosphorylation was uncoupled, either by the addition of 2,4-dinitrophenol to intact mitochondria and cyclophorase preparations, or by using damaged mitochondria, oxygen uptake with L-glutamate and pyruvate as substrates was still inhibited by glyoxylate plus oxaloacetate.

4. Glyoxylate alone also inhibited the oxidation of pyruvate, citrate, oxoglutarate and succinate in intact mitochondria. When the substrates were pyruvate or citrate the inhibition was accompanied by a consistent accumulation of citrate, but no accumulation was found when the substrate was succinate or oxoglutarate. When oxaloacetate was added in addition to glyoxylate, the accumulation of citrate increased considerably with pyruvate or citrate as substrate but only slightly with oxoglutarate or succinate.

5. Oxalomalate inhibited the oxidation of all the substrates tested, but, compared with that of glyoxylate, the inhibition was higher on citrate or pyruvate than on oxoglutarate or succinate. Glyoxylate behaved in the opposite way.

6. The inhibition by glyoxylate of the oxidation of oxoglutarate and of succinate, but not of that of citrate, was abolished by an equimolecular concentration of pyruvate.

7. The possibility that glyoxylate, besides inhibiting aconitase through the formation of oxalomalate, may interfere with some other enzymic system of the cycle is discussed.

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Studies on the Deoxyribonucleases of Bacteriophage-Infected *Escherichia coli*

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Extracts prepared from *Escherichia coli* after infection with bacteriophages T2, T4, T5 or T6 show higher deoxyribonuclease activities than do comparable extracts from uninfected bacteria (Pardee & Williams, 1952, 1953; Kozloff, 1953; Kunkee & Pardee, 1956; Crawford, 1959). Kozloff (1953) suggested that the increased activity is due to the phage causing the destruction of a minor

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