The Effect of Oxygen on Tetrathionate Reductase Activity and Biosynthesis

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SUMMARY

Cyanide (1 mM) strongly inhibited aerobic respiration, and 2,4 dinitrophenol (0.2 mM) apparently uncoupled oxidative phosphorylation, in non-growing *Citrobacter*. At these inhibitor concentrations, anaerobic tetrathionate reductase activity was not much affected. Aeration inhibited tetrathionate reductase activity; 0.2 mM-2,4 DNP did not influence oxygen inhibition, but 1 mM-KCN restored the reductase activity quantitatively. The process of aerobic respiration rather than the oxygen molecule itself therefore inhibits tetrathionate reductase activity. Induced synthesis of reductase required anaerobic conditions. Cyanide and 2,4 DNP allowed anaerobic synthesis of reductase; aeration prevented it. This effect of oxygen was abolished neither by KCN nor by 2,4 DNP. Oxygen therefore represses the synthesis of tetrathionate reductase directly.

INTRODUCTION

That some facultative anaerobic bacteria reduce tetrathionate to thiosulphate was discovered by Pollock and co-workers (Pollock, Knox & Gell, 1942; Pollock & Knox, 1943; Knox, Gell & Pollock, 1943). The reaction was quantitative:

H^+ and thiosulphate accumulated in the medium. Later, Knox & Pollock (1944) demonstrated that the enzyme responsible for the reduction of tetrathionate was both specific and inducible, the sole known substrate and inducer being tetrathionate. Tetrathionate reduction is widespread among the facultative anaerobes and has some taxonomic and diagnostic value (Le Minor & Pichinoty, 1963; Nicolle & Le Minor, 1965; Le Minor, 1967; Papavassiliou, Samarakis-Lyberopoulou & Piperakis, 1969). Discussing the possible physiological role of tetrathionate reductase in bacteria, Pollock and co-workers concluded that it may be a form of anaerobic respiration analogous to the reduction of nitrate to nitrite. This analogy was supported by the finding (Pollock & Knox, 1943) that tetrathionate reductase activity, like nitrate reductase activity (Stickland, 1931), was reversibly inhibited by aeration. Pichinoty & Bigliardi-Rouvier (1963) found that oxygen not only inhibited the activity of tetrathionate reductase but also repressed its synthesis.

Our investigation attempts to clarify the mechanism of this dual action of oxygen in washed non-growing organisms. Tetrathionate reductase is not the sole bacterial enzyme which is inhibited and repressed by oxygen (Fowler, 1951; Pichinoty, 1965; Gray, Wimpenny, Hughes & Mossman, 1966; Gray, Wimpenny & Mossman, 1966),

thus results reported here are also a contribution to the understanding of a general phenomenon called the 'oxygen effect' (Pichinoty, 1965).

METHODS

Micro-organism and cultivation. The original strain isolated by Pollock (1946) and labelled '1433', recently identified by Sedlak & Slajsova (1967) as *Citrobacter*, was grown on a reciprocating shaker at 30° in the following medium (g./l. dist. H₂O): Na₂HPO₄·12H₂O, 3·57; KH₂PO₄, 0·98; MgSO₄·7H₂O, 0·03; NH₄Cl, 0·50; traces of FeSO₄ and CaCl₂; lactose, 2·0; yeast extract Difco, 0·5; Casamino acids Difco, 0·5; bacto-peptone Difco, 0·5. The pH was adjusted to 6·8 before sterilization for 20 min. at 127°; lactose was sterilized separately. If organisms possessing tetrathionate reductase activity were required, a solution of 1·0 g. K₂S₄O₆/l. (sterilized by filtration) was added as inducer. After 15 h. the bacteria (by then in the stationary phase) were harvested, washed twice and resuspended in distilled water.

Although aerobic cultivation represses the synthesis of tetrathionate reductase (Pichinoty & Bigliardi-Rouvier, 1963), organisms containing much tetrathionate reductase can be grown as described above because the concentration of dissolved oxygen decreased during growth of the culture and was practically zero at the high bacterial concentrations reached towards the end of the exponential growth phase. When the organisms were harvested, tetrathionate was still present in the medium indicating that inducer had been present throughout growth. This mode of cultivation had the advantage of a fourfold greater yield of active organisms compared with anaerobic cultivation.

Tetrathionate reductase activity. This was measured anaerobically as follows (Pollock & Knox, 1943; Kaprálek, Pichinoty & Riegrová, 1969): A buffer solution containing a source of hydrogen and electrons (e.g. glucose) and washed bacterial cells was warmed to 37° and K₂S₄O₆ was added at time zero (*t*₀). The composition of the test systems and the concentrations of components are given with the appropriate figures. This manner of static incubation of 20 ml. of thick bacterial suspension in an 18 mm. test-tube, provided adequately anaerobic conditions and was equivalent to incubation in a vacuum in a Thunberg tube or to incubation with bubbling O₂-free nitrogen through the solution. If aerobic conditions were required, 10 vol. of air/vol. medium/min. was sparged through the system. At intervals, 1 ml. samples were transferred to 3 ml. 96 % (v/v) ethanol or into 1 ml. of 5 % (w/v) trichloroacetic acid to stop the reaction. Thiosulphate produced was then assayed iodometrically. From these data the initial velocity of tetrathionate reduction (*v*_i = μmole K₂S₄O₆ reduced/h./mg. dry wt) was calculated as the product of the first-order reaction rate constant and initial tetrathionate concentration (Kaprálék *et al.* 1969) and taken as the measure of tetrathionate reductase activity. The micro-organism used in our work did not reduce thiosulphate enzymatically, and therefore the thiosulphate found in the medium corresponded quantitatively to the tetrathionate reduced.

Induction of tetrathionate reductase. This was carried out in a similar manner to the assay above. Exact experimental conditions are mentioned under relevant figures.

Rate of oxygen uptake. This was measured using a Warburg apparatus at 37°, with air as gas phase. Results were expressed as *Q*_{O₂} (μl. O₂ consumed/h./mg. dry wt). The experiments with KCN were carried out using the method of Robbie (1946).

RESULTS

The inhibitory effect of oxygen on tetrathionate reductase activity

The inhibition of tetrathionate reductase activity by oxygen is demonstrated in Fig. 1. The inhibition disappeared immediately after aeration ceased. Nothing is known about the mechanism of this reversible inhibitory effect of oxygen. Three possibilities may be considered.

(1) Oxygen may directly and reversibly inactivate the tetrathionate reductase molecule. In this case its inhibitory effect would depend only on the concentration of oxygen in solution.

Fig. 1. Effect of aeration on the tetrathionate reductase activity of washed organisms previously grown in the presence of $K_2S_4O_6$, 0.16 M-phosphate buffer, pH 6.8; glucose 50 μ mole/ml.; $K_2S_4O_6$ 25 μ mole/ml.; chloramphenicol 50 μ g./ml.; washed organisms equiv. 1.5 mg. dry wt./ml.; final volume 20 ml. $\circ$ — $\circ$, Anaerobically; $\bullet$ — $\bullet$, aerobically; arrow indicates the time the aeration was stopped.

(2) The oxygen itself may not be the inhibitor. It may act indirectly in that electrons are preferentially transferred to it for aerobic respiration. If so, the inhibitory effect would not depend on the dissolved oxygen concentration but on the organism's Q_{O_2} .

(3) Aerobic respiration brings about oxidative phosphorylation leading to an increased rate of ATP formation and decreased steady state level of ADP. Tetrathionate respiration might thus be inhibited in a manner analogous to the aerobic inhibition of glucose fermentation (the Pasteur effect), i.e. indirectly through the ratio of ATP to ADP. If so, inhibition by oxygen would depend neither on its concentration in solution nor on the Q_{O_2} activity, but on the ability of the organisms to perform aerobic oxidative phosphorylation.

Fig. 2 shows that 2,4 dinitrophenol did not influence the tetrathionate reductase activity at concentrations < 0.25 mM, whereas from 0.01 mM it stimulated the respiratory activity as much as 40%. At such concentrations 2,4 DNP uncouples phosphorylation from oxidation and thus usually causes an increase in the rate of oxygen consumption (James, 1953; Dolin, 1961; Slater, 1963). It is probable that the inhibitor acted similarly at 0.2 mM in our experiments. This conclusion is supported by growth experiments

(Fig. 2). The aerobic growth rate with 0.2 mM-2,4 DNP was equal to the rate of anaerobic growth. Nevertheless, no concentration of 2,4 DNP tested abolished the inhibitory effect of aeration on tetrathionate reductase activity (Fig. 2, the lowest curve), so it is probable that the inhibitory effect of oxygen on reductase activity was not determined by the ability of organisms to carry out aerobic oxidative phosphorylation. Thus the activity of tetrathionate reductase seems not to be controlled by the intracellular levels of ADP and ATP.

Fig. 2. Effect of 2,4 dinitrophenol on the respiratory activity Q_{O_2} ($\mu\text{l.}/\text{h.}/\text{mg. dry wt}$) (0.16 M-phosphate buffer, pH 6.8; glucose 50 $\mu\text{mole}/\text{ml.}$; chloramphenicol 50 $\mu\text{g.}/\text{ml.}$; washed organisms equiv. 0.364 mg. dry wt/ml.), on the tetrathionate reductase activity v_t ($\mu\text{mole}/\text{h.}/\text{mg.}$) (0.16 M-phosphate buffer, pH 6.8; glucose 50 $\mu\text{mole}/\text{ml.}$; $\text{K}_2\text{S}_2\text{O}_8$ 25 $\mu\text{mole}/\text{ml.}$; washed organisms equiv. 2 mg. dry wt/ml.; chloramphenicol 50 $\mu\text{g.}/\text{ml.}$; final volume 20 ml.) and on the growth rate $c = (\log_2 x_2 - \log_2 x_1)/(t_2 - t_1)$ (h.^{-1}) ($\text{Na}_2\text{HPO}_4 \cdot 12 \text{ H}_2\text{O}$, 3.57 g.; KH_2PO_4 , 0.98 g.; $\text{MgSO}_4 \cdot 7 \text{ H}_2\text{O}$, 0.03 g.; NH_4Cl , 0.50 g.; FeSO_4 and CaCl_2 , traces; glucose, 2.0 g.; distilled water ad 1000 ml., pH 6.8). $\bigcirc$ — $\bigcirc$, Tetrathionate reductase activity anaerobically; $\bullet$ — $\bullet$, tetrathionate reductase activity aerobically; $\square$ — $\square$, growth rate anaerobically (in O_2 -free nitrogen); $\blacksquare$ — $\blacksquare$, growth rate aerobically (reciprocating shaker); $\triangle$ — $\triangle$, respiratory activity Q_{O_2} .

Fig. 3. shows that KCN (up to 1 mM) did not influence the activity of tetrathionate reductase anaerobically but strongly inhibited the aerobic respiration. However, inhibition of tetrathionate reductase activity by aeration did not occur in the presence KCN (Table 1). Figure 4 shows a direct correlation between respiratory activity and tetrathionate reductase activity in the presence of oxygen. A graphical extrapolation (Dixon, 1953) of the data of this figure gave a dissociation constant (K_i) of 0.02 mM. Figure 5 shows the instantaneous (curve 2) and reversible (curves, 3, 3') nature of the effect of cyanide.

Later experiments using carbon monoxide verified the results found with cyanide. Table 2 shows inhibition of respiration by CO: the sensitivity of aerobic respiration to CO was very low and Q_{O_2} values below 70 % of the control were not obtained.

Table 3 shows that CO similarly to KCN reduced the inhibition of tetrathionate reductase by oxygen, but only partially, in line with its partial inhibition of the aerobic respiration. Tetrathionate reductase activity itself was not influenced by CO.

Thus cyanide and CO abolished the inhibition of tetrathionate reductase activity by oxygen by inhibiting aerobic respiration. Even in the presence of dissolved oxygen the

Fig. 3. Effect of KCN on the respiratory activity Q_{O_2} $\circ$ — $\circ$ ($\mu\text{l.}/\text{h.}/\text{mg.}$) (0.16 M-phosphate buffer, pH 6.8; glucose 50 $\mu\text{mole}/\text{ml.}$; chloramphenicol 50 $\mu\text{g.}/\text{ml.}$; washed organisms equiv. 0.285 mg. dry wt/ml.) and on the tetrathionate reductase activity v_t $\bullet$ — $\bullet$ ($\mu\text{mole}/\text{h.}/\text{mg.}$) (0.16 M-phosphate buffer pH 6.8; glucose 50 $\mu\text{mole}/\text{ml.}$; $\text{K}_2\text{S}_4\text{O}_6$ 25 $\mu\text{mole}/\text{ml.}$; washed organisms equiv. 1 mg. dry wt/ml.; chloramphenicol 50 $\mu\text{g.}/\text{ml.}$; final volume 20 ml.).

Table 1. Inhibition of tetrathionate reductase activity of washed *Citrobacter* by oxygen in the presence of KCN

0.16 M-phosphate buffer, pH 6.8; glucose 50 $\mu\text{mole}/\text{ml.}$; $\text{K}_2\text{S}_4\text{O}_6$ 25 $\mu\text{mole}/\text{ml.}$; washed organisms equiv. 1 mg. dry wt/ml.; chloramphenicol 50 $\mu\text{g.}/\text{ml.}$; final volume 20 ml.

KCN mm	Tetrathionate reductase activity v_t ($\mu\text{mole}/\text{h.}/\text{mg.}$)	
	Anaerobically	Aerobically
0	9.9	0.0
0.1	11.6	9.4
1.0	11.9	8.8

tetrathionate reductase system remained active because the transport of electrons to oxygen was blocked. Thus it was not oxygen itself, but aerobic respiration (the preferential transport of electrons to oxygen) that inhibited the tetrathionate reductase activity.

The question arose whether the reverse was true: could anaerobic respiration of tetrathionate inhibit aerobic respiration? Table 4 indicates an affirmative answer: the anaerobic respiration of tetrathionate inhibited the aerobic respiration of oxygen. It

Fig. 4. Reversal by KCN of the inhibitory effect of oxygen on tetrathionate reductase activity as function of KCN concentration. $\circ$ — $\circ$, Respiratory activity Q_{O_2} ($\mu\text{l./h./mg}$): 0.16 M-phosphate buffer, pH 6.8; glucose 50 $\mu\text{mole/ml}$; washed organisms equiv. 0.361 mg. dry wt./ml.; chloramphenicol 50 $\mu\text{g./ml}$. $\bullet$ — $\bullet$, Tetrathionate reductase activity v_t ($\mu\text{mole/h./mg.}$) under aerobic conditions: 0.16 M-phosphate buffer, pH 6.8; glucose 50 $\mu\text{mole/ml}$; washed organisms equiv. 1 mg. dry wt./ml.; $\text{K}_2\text{S}_4\text{O}_6$ 25 $\mu\text{mole/ml}$; chloramphenicol 50 $\mu\text{g./ml}$; final volume 20 ml.

Fig. 5. Inhibitory effect of aeration on the activity of tetrathionate reductase and instantaneous and reversible removal of inhibition by cyanide. System 1: 0.16 M-phosphate buffer, pH 6.8; $\text{K}_2\text{S}_4\text{O}_6$ 25 $\mu\text{mole/ml}$; washed organisms equiv. 1 mg. dry wt./ml.; glucose 50 $\mu\text{mole/ml}$; chloramphenicol 50 $\mu\text{g./ml}$; aerated. System 2: the same as system 1 (i.e. aerated) but at 20 min. 1 mM-KCN added. System 3: the same as system 1 (i.e. aerated) plus 1 mM-KCN; after 20 min. the organisms were washed and resuspended in the same medium but without KCN (3'); at 40 min. aeration was stopped (3'').

is not the tetrathionate itself which inhibits, since $K_2S_4O_6$ did not affect the Q_{O_2} of the organisms (B) lacking tetrathionate reductase activity. The lowering of Q_{O_2} , though significant, was not high and did not always occur. Its manifestation depended on the source of electrons and other factors such as the difference between A and C organisms. It was apparently conditioned by the proportions of aerobic and anaerobic respiratory activities in bacteria possessing both. The decisive fact is that oxygen may not fully inhibit the tetrathionate reductase activity in aerobic conditions.

Table 2. Effect of CO on respiratory activity Q_{O_2}

The composition of liquid phase in Warburg vessel: 0.16 M-phosphate buffer, pH 6.8; glucose 50 μ mole/ml.; washed bacteria equiv. 0.31 mg. dry wt/ml.; chloramphenicol 50 μ g./ml. The rate of chemical absorption of CO in KOH is subtracted, the endogenous respiration is not subtracted. The carbon monoxide was 99.995 % pure (L'air liquide, Paris).

Composition of gas phase (%)	$\left\{ \begin{array}{l} N_2 \\ CO \\ O_2 \end{array} \right.$	—	95	—	90	—	85	—	80	—
	Air	—	—	95	—	90	—	85	—	80
	O_2	—	5	5	10	10	15	15	20	20
Aerobic respiratory activity Q_{O_2} (μ l./h./mg.)		206	127	91	169	125	200	172	202	183
Aerobic respiratory activity expressed in percentage of the control activity of (CO substituted by N_2)		—	100	71.6	100	74.0	100	86.0	100	90.6

Table 3. Effect of CO on tetrathionate reductase activity in the presence and absence of oxygen

The composition of liquid phase: 0.16 M-phosphate buffer, pH 6.8; $K_2S_4O_6$ 25 μ mole/ml.; glucose 50 μ mole/ml.; washed bacteria equiv. 1 mg. dry wt/ml.; final volume 20 ml. This solution was aerated with gaseous phases of the following composition.

The composition of gas phase (%)	$\left\{ \begin{array}{l} N_2 \\ Air \\ CO \end{array} \right.$	—	100	89.3	50	—	—	—
	No aeration	—	—	10.7	50	—	10.7	50
	CO	—	—	—	—	100	89.3	50
The composition of gas phase in partial pressure (atm.)	$\left\{ \begin{array}{l} N_2 \\ Air \\ CO \end{array} \right.$	(Anaero- bically)	1.04	0.93	0.52	—	—	—
	—	—	—	0.11	0.52	—	0.11	0.52
	CO	—	—	—	—	1.04	0.93	0.52
Tetrathionate reductase activity v_t (μ mole/h./mg.)		10.3	10.3	1.8	0.0	10.3	8.7	0.5

The inhibitory effect of oxygen on tetrathionate reductase biosynthesis

The induction of tetrathionate reductase synthesis in washed non-growing organisms is shown in Fig. 6. With oxygen, as with chloramphenicol, induction did not take place. The repressive effect of oxygen on the tetrathionate reductase synthesis was reversible: after aeration ceased, induction occurred at practically the same rate as without previous aeration. The last curve excludes the possibility that inductive synthesis of reductase occurred during aeration but that its activity was inhibited by oxygen: after aeration ceased and anaerobic conditions were established in the presence of chloramphenicol (which did not influence the activity of enzyme already formed), no tetrathionate was reduced. Oxygen therefore repressed synthesis of tetrathionate reductase. Repression by oxygen did not involve ATP formed by oxidative phosphorylation in

Table 4. *Effect of tetrathionate on the aerobic respiratory utilization of oxygen*

Warburg vessels contained: 0.16 M-phosphate buffer, pH 6.8; glucose or lactate or formate 50 μ mole/ml.; washed organisms A or B or C equiv. 0.666 mg. dry wt/ml.; chloramphenicol 50 μ g./ml.; $K_2S_4O_6$ 25 μ mole/ml. (omitted in control experiments). All values of Q_{O_2} are means from six parallel vessels. Tetrathionate reductase activity was assayed separately in a solution of the same composition. Organisms A were grown in the presence of $K_2S_4O_6$; organisms B in the absence of tetrathionate; organisms C were grown also without $K_2S_4O_6$, then washed, resuspended (2 mg. dry wt/ml.) anaerobically in a solution of phosphate buffer, pH 6.8 (0.16 M), $K_2S_4O_6$ (25 μ mole/ml.), Casamino acids Difco (1 mg./ml.) and glucose (50 mole/ml.) and incubated at 30° for 1 h. (final volume 50 ml.). Under these conditions the induction of tetrathionate reductase in resting cells occurred.

Kinds of organisms	Reductant	$K_2S_4O_6$	Respiratory activity Q_{O_2} (μ l./h./mg.)	Q_{O_2} (%)	Student's value t	Corresponding probability P	Tetrathionate reductase activity v_t (μ mole/h./mg.)	
							Anaerobically	Aerobically
A	Glucose	Absent	96 $\pm$ 5.349	100	5.16	0.007	9.9	0.8
		Present	83 $\pm$ 3.066	86				
A	Lactate	Absent	120 $\pm$ 1.673	100	7.63	0.002	6.5	1.1
		Present	108 $\pm$ 3.464	90				
A	Formate	Absent	65 $\pm$ 3.633	100	0.50	0.61	6.8	0.7
		Present	64 $\pm$ 3.225	98				
B	Glucose	Absent	70 $\pm$ 3.000	100	0.42	0.66	0.0	0.0
		Present	68 $\pm$ 2.191	97				
B	Lactate	Absent	134 $\pm$ 3.715	100	0.29	0.74	0.0	0.0
		Present	133 $\pm$ 7.681	99				
B	Formate	Absent	23 $\pm$ 1.732	100	0.00	1.00	0.0	0.0
		Present	23 $\pm$ 2.191	100				
C	Glucose	Absent	84 $\pm$ 5.020	100	0.00	1.00	4.3	0.3
		Present	84 $\pm$ 3.406	100				
C	Lactate	Absent	115 $\pm$ 5.138	100	0.93	0.40	3.9	0.3
		Present	113 $\pm$ 1.000	98				

aerobiosis since no aerobic synthesis of tetrathionate reductase took place at any concentration of 2,4 DNP (including 0.2 mM, which we showed earlier uncoupled oxidative phosphorylation from aerobic respiration) (Fig. 7). Fig. 8 shows that oxygen repression was not reversed by 1 mM cyanide which almost completely inhibited aerobic respiration (Fig. 3). It follows that aerobic respiration was not the factor inhibiting reductase synthesis: oxygen repression occurred whether aerobic respiration was present or not.

Fig. 6

Fig. 7

Fig. 6. Induction of tetrathionate reductase synthesis in washed non-growing bacteria and the repressive effect of oxygen: 0.11 M-phosphate buffer, pH 6.8; glucose 50 μ mole/ml.; washed organisms equiv. 2 mg. dry wt./ml.; $K_2S_4O_6$ 25 μ mole/ml.; final volume 20 ml. ○—○, Anaerobically; □—□, aerobically; ●—●, anaerobically + chloramphenicol 50 μ g./ml.; ▲—▲, 60 min. aerobically, then anaerobically; 60 min. aerobically, then anaerobically + chloramphenicol 50 μ g./ml.

Fig. 7. Induction of tetrathionate reductase synthesis in washed non-growing bacteria anaerobically and aerobically in the presence of 2,4 dinitrophenol: 0.16 M-phosphate buffer, pH 6.8; glucose 50 μ mole/ml.; $K_2S_4O_6$ 25 μ mole/ml.; washed organisms equiv. 2 mg. dry wt./ml.; final volume 20 ml. ○—○, Anaerobically; ●—●, anaerobically + 0.01 mM-2,4 DNP; □—□, anaerobically + 0.1 mM-2,4 DNP; ■—■, anaerobically + 0.2 mM-2,4 DNP; △—△, anaerobically + 0.4 mM-2,4 DNP; ×—×, aerobically = anaerobically + chloramphenicol 50 μ g./ml. = aerobically + 0.01 mM or 0.1 mM or 0.2 mM or 0.4 mM 2,4 DNP.

DISCUSSION

The results presented here support the idea that both terminal enzymes, the aerobic oxidase and the tetrathionate reductase, compete for electrons from a shared electron carrier:

The nature of this common carrier is unknown at present, but it is insensitive to cyanide. Aerobic respiration and oxygen, as the final electron acceptor, are preferred and aerobic respiration strongly inhibits tetrathionate respiration. A preliminary analysis of the reaction kinetics in a divergent linear chain of this type shows that behaviour in accordance with our experimental findings would be exhibited by a system in which either the concentration of aerobic oxidase were tenfold to 100-fold greater than the concentration of tetrathionate reductase (with the same Michaelis

Fig. 8. Induction of tetrathionate reductase synthesis in washed non-growing bacteria anaerobically and aerobically in the presence of KCN: 0.11 M-phosphate buffer, pH 6.8; glucose 50 μ mole/ml.; $K_2S_4O_6$ 25 μ mole/ml.; washed organisms equiv. 2 mg. dry wt/ml.; final volume 20 ml. ○—○, Anaerobically; ×—×, anaerobically + 0.1 mM-KCN; △—△, anaerobically + 1 mM-KCN; ●—●, aerobically = anaerobically + chloramphenicol 50 μ g./ml. = aerobically + 0.1 mM or 1 mM-KCN.

constants of both toward the common electron donor), or the Michaelis constant of aerobic oxidation toward common donor is tenfold to 100-fold lower than the Michaelis constant of tetrathionate reductase at the same concentrations of both enzymes. As the rates of oxygen consumption and of tetrathionate reduction measured separately and expressed in terms of electron equivalents transferred/h./mg. dry wt did not differ substantially (see Fig. 3), the second possibility is more probable. Nevertheless, the degree of mutual inhibition may depend on the relative concentrations of the competing terminal enzymes (Table 4). The mode by which oxygen inhibits tetrathionate reductase activity implies that competitive electron acceptors other than oxygen should decrease the rate of tetrathionate reduction.

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