analysis, analogous sections may be found on DLTC and LDTC in practically all records under aerobic conditions (even, for example, on R 478). Under nitrogen, the LDTC has a character of a rapid monophasic decay and it is never found to be symmetrical with the DLTC.

In a previous communication, the author has suggested that the shape of the aerobic LDTC records results from the (pseudo)monomolecular liberation of oxygen from an intermediary product present at the moment the light is shut off. To explain the two inflections or minima commonly observed on the LDTC, two oxygen-consuming processes were assumed to take place at the beginning of the dark period. This type of explanation, however, seems rather inadequate, if the striking symmetry of the LDTC and DLTC is taken into account. It would be strange if by mere chance similar time constants were to govern both the start of the complicated reaction chain in photosynthesis and the single reaction of oxygen liberation from an intermediary product. It seems more plausible that, in some way or other, the LDTC reflect changes in reservoir sizes of the same intermediates and variations in rates of the same reversible reactions, which are reflected by the DLTC when the opposite transition from dark to light takes place.

Before a reasonable interpretation of the phenomena observed can be attempted, more data must be collected. In particular, parallel determinations of oxygen exchange by a dropping mercury electrode and by the method of BLINKS AND SKOW are planned. It is the disadvantage of the latter method that it fails to give a clear discrimination between net oxygen production and uptake. Consequently, the point of basic interest cannot be established, *i.e.*, which sections of the records fall above the compensation point and which are below it. To know this is obviously of great importance for an adequate interpretation of the LDTC and for the correct evaluation of interactions existing between photosynthesis and respiration. That such interrelations influence the shape of aerobic LDTC records is suggested by the striking difference between aerobic and anaerobic light-dark transition curves.

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Inhibition of citrate oxidation by glyoxylate in rat liver homogenates

The mechanism of the inhibition produced by glyoxylate on a respiring tissue suspension, first shown by KLEINZELLER[1], has been investigated by studying the action of very small amounts of glyoxylate on the oxidative behaviour of the most important metabolites related to the tricarboxylic acid cycle.

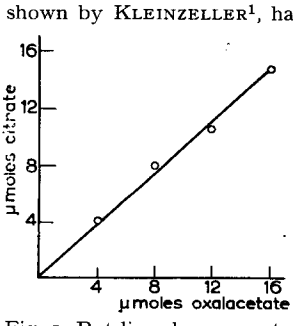

Fig. 1. Rat liver homogenate 1:10; glyoxylate 0.001 *M*; NaF 0.01 *M*.

For this purpose 0.001 *M* glyoxylate was added to a rat liver homogenate incubated in the presence of citrate, *cis*-aconitate, *iso*citrate, *α*-ketoglutarate, succinate, fumarate, malate, oxalacetate and pyruvate, each at a final concentration of 0.002 *M*. The final volume of the incubation mixtures was always 4.0 ml; the concentration of the homogenate was 1:10 (prepared in Krebs' phospho-saline medium free of Ca^{++} and with the addition of 0.025 *M* Mg^{++} and 0.01 *M* F^-). The incubation was for 60 min at 38° C in Warburg vessels of about 15 ml capacity; O_2 gas phase; pH 7.4. Another experiment was carried out under the same conditions with 0.001 *M* glyoxylate and differing concentrations of oxalacetate as shown in Fig. 1.

In all the experiments the oxygen uptake was followed manometrically, and at the end of the incubation citrate was determined

by the method of PUCHER[2], after deproteinization with 0.5 ml 30 % trichloroacetic acid, and by the methods of ETTINGER *et al.*[3] after deproteinization with 2 ml 5 % tungstic acid. All the results (see Table I and Fig. 1) are reported as μatoms oxygen uptake in 60 min μmoles citrate found in each vessel.

TABLE I

EFFECT OF GLYOXYLATE ON OXYGEN UPTAKE AND CITRATE ACCUMULATION

Reaction vol., 4 ml. 60 min. 38° C.

Substrate (8 μmoles)	*Glyoxylate (4 μmoles)*	*μatoms oxygen*	*Inhibition %*	*Citrate formed*	
				μmoles	*Δ μmoles*
None	—	100	0	0	0
	+	105		0	
Citrate	—	150	6.7	0.92	0.64
	+	140		1.56	
cis-Aconitate	—	167	2.2	1.20	0.50
	+	164		1.70	
*dl-iso*Citrate	—	78	4.0	0.80	0.80
	+	71		1.60	
α-Ketoglutarate	—	150	6.4	1.12	0.38
	+	140		1.50	
Succinate	—	147	8.7	1.26	1.74
	+	134		3.00	
Oxalacetate	—	129	44.0	0.90	9.30
	+	72		10.20	
Pyruvate	—	151	10.0	1.90	1.00
	+	134		2.90	
Fumarate	—	161	7.0	1.95	1.15
	+	150		3.10	
Malate	—	141	8.6	2.44	0.60
	+	128		3.04	

The results in Table I show that the addition of 0.001 *M* glyoxylate, while it did not modify the basal (endogenous) oxygen uptake of the homogenate, produced a small but constant inhibition of the oxidation of all the tested substrates of the tricarboxylic acid cycle, except for oxalacetate, where the inhibition was much higher. In addition, there was a parallel increase in the citrate production, which was again much higher in the case of oxalacetate. In this case it appeared that all the oxalacetate added was recovered as citrate.

Fig. 1 shows the accumulation of citrate with glyoxylate and increasing oxalacetate concentrations. The citrate formed appears to increase proportionately to the oxalacetate added. When, on the other hand, oxalacetate was incubated with increasing glyoxylate concentrations from 0.001 to 0.004 *M*, the accumulated citrate corresponded roughly to the oxalacetate and was completely independent of glyoxylate.

The dependence of citrate accumulation on oxalacetate and on only a catalytic amount of glyoxylate suggests the following mechanism. Oxalacetate may react in two different ways: (1) with acetyl CoA of the tissue to give rise to citrate and (2) with glyoxylate to form an inhibitor of citrate metabolism accounting for the accumulation and the depressed oxygen uptake. This inhibitor may well be a new tricarboxylic acid, oxalomalate, which differs from citrate by having a $-CO\cdot COOH$ group instead of $-CH_2\cdot COOH$ and could be an effective competitive analogue.

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