separated from the heme-proteins to be formed[2]. Similarly, the active fraction in Table I may have a right to be called an enzyme, although the naming may be postponed until the exact mechanism of the reaction is elucidated.

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Lactic oxidase *Tetrahymena pyriformis*: The over-all reaction mechanism

In a previous communication, the authors described an unusual lactic oxidase in the protozoan *Tetrahymena pyriformis*[1]. It was reported that pyruvate was the end-product of L-lactate oxidation, and that in crude preparations the enzyme did not (a) exhibit a requirement for DPN with O_2 as electron acceptor, (b) reduce DPN or oxidize DPNH in the presence of lactate or pyruvate, or (c) suffer appreciable inhibition by cyanide, amytal, 2-hydroxy-3-(2-methyloctyl)-1,4-naphthoquinone, lecithinase A (crotoxin), or an inhibitor obtained from the protozoan[2]. Since the latter 5 agents markedly inhibit the DPN-linked β-hydroxybutyric and glutamic oxidases—and except for amytal in relatively low concentration, succinic oxidase as well—it was apparent that, unlike the conventional lactic dehydrogenases, the *Tetrahymena* enzyme is not joined to the terminal electron-transport chain(s) involved in the oxidation of DPNH and succinate. This note outlines evidence for the mechanism of the over-all lactic oxidase reaction and presents some additional properties of the crude enzyme. Methods for the growth and collection of the protozoa and the preparation of cell-free homogenates have been described[3,4]. Lactic oxidase activity was measured by following O_2 consumption at 30° as indicated previously[1].

KEILIN AND HARTREE[5] have demonstrated that the addition of ethanol to such primary oxidizing systems as xanthine oxidase, uricase, glucose oxidase, and D-amino acid oxidase, already containing catalase, doubled the total O_2 uptake by these systems, since H_2O_2 formed in the primary reactions was utilized for the secondary or coupled oxidation of ethanol to acetaldehyde instead of being decomposed catalatically, liberating O_2. Similar experiments have been carried out with the lactic

Abbreviations: DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide.

oxidase of *Tetrahymena*. A typical one, illustrated in Fig. 1, shows that in the presence of ethanol the complete oxidation of L-lactate by *T. pyriformis* S homogenate (which contains catalase) is accompanied by twice the theoretical O_2 uptake represented by

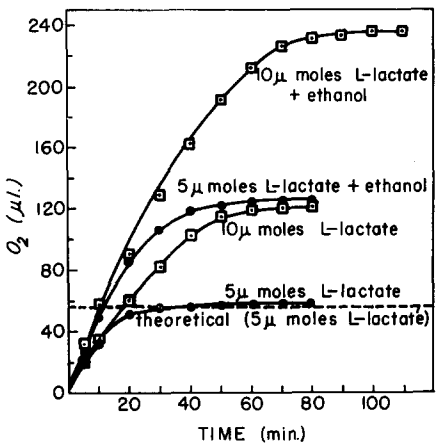

Fig. 1. The oxidation of L-lactate in the presence or absence of ethanol by *T. pyriformis* S homogenate. Pairs of vessels contained 1.0 ml 0.1 *M* K_2HPO_4–KH_2PO_4 buffer, pH 7.1, in the main chambers; Na lactate in the amount indicated and 75 mg ethanol, where present, in the side arms; 0.2 ml 20% KOH in the center wells. After 5 min equilibration, the vessels were tipped and the taps closed 2.5 min later. Final readings were corrected by extrapolation to the tipping time. Vol., 3.0 ml; gas phase, air.

reaction (1). Hence, it is evident that the protozoan enzyme catalyzes the oxidation of lactate to pyruvate and H_2O_2 in accordance with reaction (2).

$$CH_3CHOHCOOH + 1/2O_2 \rightarrow CH_3COCOOH + H_2O \quad (1)$$

$$CH_3CHOHCOOH + O_2 \rightarrow CH_3COCOOH + H_2O_2 \quad (2)$$

The postulated reaction for the oxidation of lactate is further verified by the chemical balance data given in Table I. Without ethanol, the oxidation proceeds to completion with approximately 2 moles of pyruvate produced per mole of O_2 consumed, as reported[1]. With ethanol, the amount of pyruvate produced is unchanged but the net O_2 consumption is doubled. After completion of the manometric portion of the experiment summarized in Table I, the vessel contents were examined for aldehyde using a spot test involving the oxidation of *p*-phenylenediamine by H_2O_2 and for acetaldehyde by a spot test with morpholine and sodium nitroprusside[6]. Positive results were obtained with the contents of those vessels which had originally received lactate, ethanol, and enzyme. Finally, the increase in O_2 consumption of *Tetrahymena* lactic oxidase in the presence of such catalase inhibitors as hydroxylamine, azide, and cyanide is consistent with the conclusion that H_2O_2 is formed when lactate is oxidized.

The lactic enzyme of *Tetrahymena* thus behaves like an aerobic dehydrogenase, reacting directly with molecular O_2 without the participation of the cytochromes and reducing it to H_2O_2. By analogy with such H_2O_2-generating systems as xanthine oxidase, D-amino acid oxidase, glucose oxidase, and glycolic oxidase, it is likely that the lactic oxidase is a flavoprotein, perhaps similar to the purified lactic oxidase

TABLE I

OXYGEN CONSUMPTION AND PYRUVATE PRODUCTION FROM LACTATE BY *T. pyriformis* HOMOGENATE IN PRESENCE OR ABSENCE OF ETHANOL

The experimental conditions are given in Fig. 1. At the end of the reaction, trichloroacetic acid was added to all vessels and the amount of pyruvate present in the clear supernatants after centrifugation was determined by the method of KOEPSELL AND SHARPE[8].

L-Lactate added	O_2 consumed		Pyruvate produced	
	No ethanol	ethanol	No ethanol	ethanol
μmoles	μmoles		μmoles	
5.01	2.60	5.26	4.54	4.48
10.02	5.09	10.40	9.62	9.33

of *Lactobacillus delbreuckii* which reacts directly with O_2, flavins acting as hydrogen carriers[7].

It is worth noting that under conditions where relatively large amounts of ethanol (150 mg) do not inhibit lactic oxidase, the activities of the succinic[3] and β-hydroxybutyric oxidase systems are decreased 75–90 %. Antimycin (20 μg) and 2-heptyl-4-hydroxyquinoline N-oxide (20–40 μg) have no effect on *Tetrahymena* lactic oxidase, but 20 μg of the oxide inhibit succinic oxidase 60–70 % without affecting succinic dehydrogenase. Atabrine ($3 \cdot 10^{-3}$ *M*) inhibits lactic oxidase only 10 % but decreases the activities of the succinic and β-hydroxybutyric oxidase systems 70 and 50 %, respectively, without affecting succinic and β-hydroxybutyric dehydrogenases. At 10^{-2} *M*, salicylaldoxime and diethyldithiocarbamate inhibit lactic oxidase 20 and 70 %, respectively, while 10^{-3} *M* *p*-chloromercuriphenyl sulphonic acid completely inhibits the enzyme.

A detailed account of this and other work on *Tetrahymena* lactic oxidase will be published elsewhere.

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