Cyclophorase System XXIV. Oxidation of Pyruvate to Acetate in Rabbit Heart Muscle.* (19376)

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In the cyclophorase-mitochondrial (CM) system pyruvate is oxidized to carbon dioxide and water by way of the citric acid cycle(1). The initial process is the oxidative decarboxylation of pyruvate to a C_2 fragment which in the form of acetyl CoA enters the citric acid cycle by way of condensation with oxalacetate(2). In a recent review(3), it has been stated that homogenates such as those of heart muscle do not remove pyruvate aerobically, unless a dicarboxylic acid is present. This statement is based on the early studies of Ochoa(4), in which it was shown that pyruvate is not oxidized when incubated with dialyzed preparations of minced cat heart muscle.

In view of the fact that a pyruvic oxidase isolated from pigeon breast muscle or pig heart can convert pyruvate quantitatively to acetate and CO_2(5), it seemed logical to expect that the direct oxidation of pyruvate to acetate should also be demonstrable in CM preparations or in homogenates of rabbit heart muscle.

Experimental. The pertinent experimental details have already been covered in the experimental sections of the preceding two communications(6,7).

The estimation of pyruvate and acetate was carried out by the methods respectively of Friedemann and Haugen(8), and of Black(9).

Results. While studying the oxidation of pyruvate in the CM preparations of rabbit heart muscle, we observed that in the presence of malonate, one atom of oxygen was absorbed for each molecule of pyruvate metabolized. In the absence of malonate, approximately 5 atoms of oxygen were absorbed per molecule of pyruvate metabolized (Table I). Thus, depending upon the presence or absence of malonate, the following reactions may be proposed:

TABLE I.
Effect of Malonate on Pyruvate Oxidation.

Malonate present, μM	Oxygen utilized, μatoms	Pyruvate utilized, μM	O/pyruvate
0	71.4	14.2	5/1
15	15.5	13.4	1/1

Complete system consisted of 1 ml of enzyme preparation, .2 ml of .1 M $MgCl_2$, .2 ml of .1 M phosphate buffer, pH 7.4, .3 ml of .01 M adenosine-5-phosphate, 20 μM of lithium pyruvate, .2 ml of 6N KOH in center well with filter paper strip. Vessels gassed with 100% O_2 for 2 min, and equilibrated at 38°C for 5 min before taps closed.

TABLE II.
Effect of Malonate on Pyruvate Metabolism.

Malonate present, μM	Oxygen utilized, μatoms	Pyruvate utilized, μM	Acetate formed, μM	CO_2 formed, μM
50	17.6	18.1	18	17.2

Complete system: see Table I.

(1) $CH_3COCOOH + 0 \longrightarrow CH_3COOH + CO_2$,
(2) $CH_3COCOOH + 5O \longrightarrow 3CO_2 + 2H_2O$.

In Table II, additional data from a typical experiment are provided to establish that the requirements of equation (1) are satisfied when pyruvate is oxidized in presence of malonate. The mechanism by which malonate prevents the condensation of the C_2 fragment with oxalacetate is not as yet decided. Either the formation of oxalacetate from pyruvate, or the condensative reaction itself might be the site of action of malonate.

In an attempt to reduce to a minimum any oxalacetate which might arise from endogenous substrate, the enzyme preparation was subjected to dialysis (150 minutes) at 3-5°C against 100 volumes of 0.12 M potassium chloride—0.007 M magnesium chloride (changed 5 times). Under these conditions

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TABLE III. Pyruvate Metabolism After Dialysis of Enzyme Preparation.

Substrate	Oxygen utilized, μatoms	Pyruvate utilized, μM	Acetate formed, μM	CO_2 formed, μM
20 μM pyruvate	19.5	20.1	20.2	17.6
+ 5 μM fumarate	72.3*			

Complete system: see Table I.
* Fumarate blank subtracted.

the CM preparation, even in absence of malonate, converts pyruvate to acetate (Table III).

A comparable situation exists in the CM system of rat liver where it has been shown by Lehninger(10) that pyruvate is either oxidized directly to CO_2 and H_2O, or in absence of condensing partner (*e.g.* in presence of malonate), is converted quantitatively to acetoacetate. The C_2 fragment, if it is not condensed with oxalacetate, can condense with another C_2 fragment to form acetoacetate. This multiple pathway for pyruvate in liver has been studied extensively by Recknagel and Potter(11).

Discussion. The oxidation of pyruvate by the pyruvic oxidase of animal tissues has been shown by Jagannathan and Schweet(12) to lead to the stage of acetyl CoA, since the product is capable of acetylating sulfanilamide. Acetyl CoA can either condense with oxalacetate to form citrate, as has been shown by Ochoa and Stern(2), or it can be decomposed into acetate and CoA, as shown by Gergely and Hele(13). The latter deacylation reaction is so much slower than the former, that it plays a negligible role except when the condensative reaction is blocked.

Summary. Pyruvate is oxidized to CO_2 and H_2O by the cyclophorase-mitochondrial system of rabbit heart. Under appropriate conditions, *e.g.* by blocking with malonate or by prolonged dialysis of the preparation, pyruvate is oxidized to acetate and carbon dioxide, and the C_2 unit formed does not enter the citric acid cycle by condensation with oxalacetate.

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The Origin of Bradykinin. (19377)

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Bradykinin is a hypotensive and smooth-muscle stimulating substance which is formed from the globulin fraction of plasma when acted upon by proteolytic enzymes such as fibrinolysin, trypsin or snake venom. This substance, discovered by Rocha e Silva, Beraldo, and Rosenfeld(1), is characterized as follows: a) it causes a slow contraction of guinea pig, rat, and rabbit intestine which is not prevented by antihistaminics or atropine,

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