METABOLISM OF CARDIAC MUSCLE

IV. UTILIZATION OF PYRUVATE AND DL-LACTATE BY DUCK HEART*

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There is good evidence that pyruvate and lactate are important sources of energy for the intact heart. Early studies by Himwich et al. (1) in the open chest dog and by McGinty and Miller (2) in the heart-lung preparation showed that lactate was removed from the coronary blood during its perfusion of the myocardium. Subsequent studies by Braun-Menendez et al. (3) with the isolated circulating heart showed that blood pyruvate was also extracted by the intact heart. More recent investigations of myocardial metabolism in the intact, lightly anesthetized dog (4) and in man (5) by means of cardiac catheterization have confirmed and extended the previous work. There has been, however, no systematic study of lactate and pyruvate metabolism in cardiac muscle slices, although there have been a few isolated observations of the effect of these substrates upon respiration in slices (6–10) and homogenates (11). In view of the interest in this laboratory in studying the effect of vitamin deficiencies upon cardiac metabolism (12–14) in vitro, it was thought important to document the effect of certain variables upon the utilization of pyruvate and lactate in normal heart slices. Later studies in this laboratory have dealt with the synthesis of radioactive L(+) and D(−)-lactic acids, as well as with the metabolism of these isomers in animal tissues (15).

The present paper is devoted to a study by the Warburg technique of the metabolism of pyruvate and dl-lactate in slices of heart ventricle

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from normal ducks. The effects of time of incubation and substrate concentration upon the utilization of substrate and total respiration are determined. Two radioisomers of pyruvate, tagged with $^{14}C$ in the carbonyl and carboxyl groups, respectively, are employed in certain of these studies in order to ascertain the rate of oxidation of pyruvate as a function of concentration and determine the degree to which added pyruvate contributes to the oxygen consumption of the cardiac slice. It has been possible to derive a formulation describing the rate of decay of metabolic activity in heart slices incubated in the presence of pyruvate as a function of time.

EXPERIMENTAL

White Pekin ducklings were placed in heated, raised bottom cages when 1 day of age and fed an adequate purified diet (12) ad libitum for 2 to 3 weeks at which time they weighed 250 to 450 gm. The ducks were sacrificed by decapitation, the hearts quickly excised and chilled in cracked ice, and slices of ventricle prepared. For measurements of respiration, the direct method of Warburg was used with $O_2$ as the gas phase at 37°. 3.0 ml. of medium with one slice of heart muscle, 10 to 15 mg. of dry weight, in the main chamber and 0.2 ml. of 10 per cent KOH in the center well were employed. The details of the procedure and the composition of the medium have been previously described (13).

The substrates used in these experiments were redistilled non-isotopic pyruvic acid crystallized as the sodium salt (14), two $^{14}C$ radioisomers of pyruvate labeled in the carbonyl and carboxyl groups, respectively, and also prepared as the sodium salts (14), and non-isotopic commercial (analytical reagent) DL-lactic acid, depolymerized by boiling for 10 minutes, titrated with standard alkali, and made up in phosphate-saline medium to the required molarity. The initial concentration of lactate or pyruvate varied from 2 to 40 mM per liter. In a few experiments the pyruvate was added from the side arm of the flask by tipping at intervals of from 1 to 20 minutes after the addition of tissue.

At the end of the period of incubation, reactions were stopped by addition of 0.2 ml. of 100 per cent trichloroacetic acid. Pyruvate and lactate were determined on the protein-free filtrate by the methods previously employed (13). In the experiments with tagged pyruvate, the $^{14}CO_2$ captured in the center well was precipitated as $\text{Ba}^{14}C\text{O}_3$ and counted with an end window Geiger-Müller tube. Initial activities of added substrate were determined by wet combustion and counted in the same way (14). Unless otherwise specified, the results of these determinations are expressed in terms of metabolic quotients ($Q$), i.e., the number of microliters of metabolite or gas formed or lost per mg. of dry weight per hour.
(22.4 μl. = 1 μM). In this way all values are in molar proportion to the \( Q_{O_2} \). \( Q_{C1+O_2} \) was calculated as previously described (13).

**Results**

**Effect of Time on Substrate Utilization**

A feature of the metabolism of the surviving tissue slice is the prone-ness of \( Q_{O_2} \) and other parameters of metabolic activity to decrease with increasing time of incubation. This is well illustrated in Fig. 1 for duck heart slices by the time curves of pyruvate and lactate utilization. In the case of lactate, the uptake of substrate was initially rapid and came to a complete standstill at about 40 minutes. Keto acid accumulation was negligible.

In the case of pyruvate the rate of disappearance was initially rapid and then progressively less rapid. In the absence of substrate, it may be seen that lactate production from endogenous metabolites is rapid during the first 5 minutes of incubation, as indicated by a rise in lactate from 17 μM to 100 μM per gm. of dry weight in the slice after 5 minutes of incuba-
tion. Thereafter, up to 240 minutes, the lactate concentration gradually declines again.¹

In the presence of added pyruvate, the lactate formation during the first 5 minutes is 25 μM per gm. of dry weight greater than without substrate, the additional lactate assumed to have been derived from the added pyruvate by reduction. In contrast to the experiments in which no substrate is added, the lactate level reached in 5 minutes in the presence of pyruvate does not change during the remaining 235 minutes of the experiment. It seems reasonable that the explanation for this is that lactate at this concentration (about 0.8 mM per liter) is not oxidized in the presence of a 4- to 5-fold excess of pyruvate.

![Diagram](image)

**Fig. 2.** The effect of time of incubation of heart slices upon the metabolic quotients for pyruvate and DL-lactate with pyruvate or lactate initially present at 5 mM per liter. The data from which these were calculated as cumulative quotients are shown in Fig. 1. The same symbols are used.

When the cumulative changes in lactate and pyruvate plotted in Fig. 1 are expressed in terms of metabolic quotients and plotted as a function of time, the plots shown in Fig. 2 are obtained. The rapid fall in the rates of substrate utilization with time are striking, particularly for the early periods of the experiments. The curves for lactate uptake illustrate very well the artifactitious character of the metabolic quotient as a function of time, since positive values are obtained after uptake has ceased (60 minutes). The values for oxygen consumption obtained in these experiments are shown in Fig. 3. The respiration of slices of duck heart ventricle was much better sustained with pyruvate as added substrate than with lactate or no added substrate.

¹The initial burst in lactic acid formation is thought to be due to rapid glycolysis induced by increasing the temperature of the preparation in the presence of relative anoxia, since equilibration of the unoxygenated medium with oxygen is occurring during this initial interval. As the oxygen tension is raised in the medium, the conditions required for glycolysis are abolished and thereafter oxidative removal of the accumulated lactate occurs.
A mathematical formulation which would describe the rate of decline in pyruvate utilization and oxygen consumption in the cardiac muscle slice incubated for various lengths of time was sought. After trying various Q-t plots empirically, it was found that log-log plots of net $-Q_{\text{pyruvate}}$ (i.e., conversion of pyruvate to non-lactate products (12)) and $Q_{O_2}$ (in the presence of pyruvate) were linear and could be expressed as a linear equation; viz., $\log Q = m \log t - \log K$ (Fig. 5). If one solves such a linear equation for its antilogarithmic values, one obtains generically

$$Q = k t^m$$

where $Q = Q_{O_2}$ or net $-Q_{\text{pyruvate}}$, $K$ = constant which is the antilog of the $y$ intercept on the log-log plot or the value for $Q$ at 1 minute, $t$ = total time of incubation in minutes, $m$ = slope of the curve. Values for $m$ and $k$ were obtained from the experimental data on the linear plot by the method of least squares and the following equations obtained for duck heart ventricle slices.

$$Q_{O_2} = -33.0 t^{0.25} \pm 0.01$$

$$\text{Net } -Q_{\text{pyruvate}} = -67.1 t^{0.51} \pm 0.03$$

Correlation coefficients for the net $-Q_{\text{pyruvate}}$ and $O_2$ values, respectively, were 0.94 and 0.60. Similar values were obtained with rat heart slices, although in general the quotients were 50 per cent or more higher (16).
It is possible, further, to determine the rate of pyruvate utilization at any time. If one plots the cumulative data for net pyruvate disappearance shown in Fig. 1 as a function of time on a log-log plot, a straight line is obtained whose equation is 

\[
\log (\text{pyruvate uptake in micromoles per gm. of dry weight}) = 0.43 \log t + \log 46.
\]

By taking the antilogarithms of this equation one obtains

\[
\text{Pyruvate uptake (µM per gm. dry weight)} = 46t^{0.43}.
\]

The first derivative of this equation will give the rate of pyruvate uptake per unit of time at any time; viz.

\[
\frac{d \text{µM per gm. weight}}{dt} = k \times m \times t^{-m}.
\]

Since \(Q = (60 \times d \text{ microliters per mg. of dry weight})/dt\), \(Q\) can be substituted for the \((d \text{ micromoles per gm. of weight})/dt\) by modification of the constant; i.e., \(K = (k \times 22.4 \times 60 \times m)/1000\) to yield

\[
Q = 27t^{-0.87}
\]

which describes a line below that of the observed \(Q\) and parallel to it as shown in Fig. 4. From these data it appears that the rate of decay of the pyruvic acid oxidase system of heart slices in the presence of added pyruvate is a logarithmic function of the time of incubation at 37\(^\circ\).

**Effect of Concentration of Substrate upon Substrate Utilization and Oxygen Consumption**

The effects of increasing the concentrations of lactate and pyruvate upon their respective rates of disappearance in cardiac muscle slices and the corresponding oxygen uptakes are shown in Figs. 5 and 6. Oxygen consumption was stimulated with both of these substrates as their concentration in the medium was raised to 5 mM per liter and thereafter remained constant despite the increased disappearance of substrate. These facts suggest that the removal of these substrates at high concentrations is largely non-oxidative.

**Effect of Increasing Concentrations of Radioactive Pyruvate upon Recovery of C\textsuperscript{14}O\textsubscript{2} from Carboxyl- and Carbonyl-Labeled Radioisomers**

The rates of C\textsuperscript{14}O\textsubscript{2} recovery from heart slices incubated with C\textsuperscript{14}-carboxyl and C\textsuperscript{14}-carbonyl-labeled pyruvate at concentrations varying from 2 to 40 mM per liter are plotted in Fig. 6. It has been pointed out previously (14) that the difference between net \(-Q_{\text{pyruvate}}\) and \(Q_{\text{C\textsuperscript{14}O\textsubscript{2}}}\) (carboxyl), i.e., the conversion of pyruvate to non-lactate products without loss of CO\textsubscript{2}, probably represents anabolism of pyruvate in the direction of car-
Fig. 4. Log-log plot of \(Q_o_2\) and net \(-Q_{pyruvate}\) in duck heart ventricle slices as a function of time. The \(Q_o_2\) values obtained with pyruvate at 5 mM per liter as substrate are plotted as \(\circ\) in Curve C. The cumulative net \(-Q_{pyruvate}\) values obtained experimentally are plotted at the times marking the end of the period of incubation as \(\bullet\) in Curve A. The values for instantaneous net \(-Q_{pyruvate}\) at time \(t\) obtained by differentiating the equation for Curve A are plotted as \(\times\) in Curve B.

Fig. 5. The effect of concentration of DL-lactate upon the uptake of lactate and the \(Q_o_2\) in duck ventricle slices. \(\times\), \(-Q_{lactate}\) values; \(\circ\), \(Q_o_2\) values; and \(\bullet\), \(Q_{keto}\) (accumulation of keto acids).

Fig. 6. The effect of concentration of pyruvate upon the net uptake of pyruvate, \(Q_o_2\), and production of \(C^{14}O_2\) from carbonyl- and carboxyl-labeled pyruvate by duck heart ventricle slices. The substrate was initially present in the medium and the total period of incubation at 37\(^\circ\) was 75 minutes. \(\triangle\), values for net \(-Q_{pyruvate}\), and \(\circ\), those for \(Q_o_2\). \(\bullet\), values for recovery of \(C^{14}O_2\) from carbonyl-labeled pyruvate, and \(\times\), \(C^{14}O_2\) from carboxyl-labeled pyruvate. The amount of oxygen consumption associated with the combustion of added substrate is represented by \(\square\).
bohydrate synthesis (via the reactions of glycolysis). In these experiments at 5 mM per liter of pyruvate, 43 per cent of the pyruvate which disappeared did so without decarboxylation, at 10 mM per liter the fraction was 50 per cent, at 20 mM per liter it was 61 per cent, and at 40 mM per liter it was 71 per cent. At this latter concentration, no extra acetate, acetoacetate, acetoin, diacetyl, or glycogen appeared to account for the anabolic fraction. As the concentration of pyruvate increased in the medium, the amount which disappeared non-oxidatively was proportionately greater.

Certain other interesting conclusions may be drawn from these data with regard to the fraction of pyruvate oxidized. If a steady state among the intermediates of the Krebs tricarboxylic acid cycle is assumed, the amount of oxygen associated with the combustion of added pyruvate from the recovery of C\textsuperscript{14}O\textsubscript{2} from the two radioisomers of pyruvate may be calculated (14). The value for oxygen consumption due to the oxidation of added pyruvate at concentrations between 2 and 40 mM per liter is plotted in Fig. 6 as a dotted line. If one compares the line for $Q_{O_2}$ with this dotted line, it may be seen that at 5 mM per liter the contribution of oxidized pyruvate to the total oxygen consumption of the slice was 45 per cent, at 10 mM per liter it was 70 per cent, at 20 mM per liter it was 90 per cent, and at 40 mM per liter it was 93 per cent. It appears obvious from these data that, as the concentration of pyruvate was raised, more of added substrate and less of endogenous substrate in the heart slice was burned until at 40 mM per liter nearly the total oxygen consumption of the slice was accounted for by the oxidation of added pyruvate.

In connection with the old question of the interpretation of stimulations in $Q_{O_2}$ as a result of adding substrate to a tissue slice, it may be pointed out that at 2 mM per liter of pyruvate the oxygen used in the combustion of added pyruvate was equal to the degree of stimulation of the $Q_{O_2}$ above the endogenous value, while at 40 mM per liter the oxygen used in the combustion of added pyruvate was equal essentially to the entire $Q_{O_2}$.

**DISCUSSION**

Rühl (17) showed that lactate utilization in the heart-lung preparation was a function of the lactate concentration in the arterial blood and our experiments confirm this relationship for slices of ventricle *in vitro*. Other workers have studied both pyruvate (3) and lactate (2, 17) extraction in the heart-lung preparation and have obtained values very close to those obtained by us (at comparable concentrations of substrate) with slices *in vitro*. Goodale, Olson, and Hackel (5) have observed that extraction of lactate and pyruvate by the human myocardium *in vivo* is a function of
the arterial concentration of these substrates and that the rate of extraction at various arterial concentrations is comparable to that obtained in the heart-lung preparation and in the heart muscle slice. The rate of change in the O₂ consumption and utilization of pyruvate by the slice is a logarithmic function of time, and, although the log-log plot cannot be extrapolated indefinitely, the values in O₂ consumption observed at very short intervals of time compare favorably with those observed in vivo (4, 18).

With regard to the disposition of added pyruvate in cardiac slices, it has been pointed out by Olson and Stare (14) that, when carboxyl-labeled pyruvate at 5 mM per liter is tipped into flasks containing cardiac muscle slices respiring in phosphate-saline, only 50 per cent of the pyruvate which disappears to non-lactate products is decarboxylated; the remaining 50 per cent is metabolized most likely via phosphopyruvate to carbohydrate precursors. The results presented herewith confirm and extend this finding. It is noteworthy that at 40 mM per liter as much as 75 per cent of the net pyruvate disappearance can be accounted for in the anabolic fraction and this is greatly reduced by the addition of inhibitors of glycolysis such as arsenite and fluoride (19).

It also seems clear from the isotope experiments that a fraction of the pyruvate which disappears is oxidized to completion in the cardiac muscle slice. This is in opposition to the conclusion of Bernheim and Bernheim (20) who found a stimulation in Q₁₀₂ when pyruvate was added to cardiac slices and after considering the CO₂/O₂ exchange data inferred that the oxidation of pyruvate in cardiac slices stopped at the acetate stage.

With regard to the competitive inhibition of endogenous substrate oxidation by added pyruvate, it is of interest that, although the Q₁₀₂ remains constant at concentrations of pyruvate ranging from 5 to 40 mM per liter, the contribution of added pyruvate to the total oxygen consumption rises from 45 to 93 per cent.

**SUMMARY**

1. The rate of utilization of pyruvate and lactate in cardiac muscle slices is a function of the initial concentration of substrate. Both oxidative and non-oxidative reactions account for the utilization, the non-oxidative ones being more prominent at high concentrations of substrate.

2. The rate of decline in utilization of pyruvate by cardiac muscle slices is a logarithmic function of time.

3. Labeled pyruvate is oxidized to completion in the heart slice and may at high concentrations (40 mM per liter) account for practically the entire oxygen consumption of the slice.
BIBLIOGRAPHY

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IV. UTILIZATION OF PYRUVATE AND
dl-LACTATE BY DUCK HEART
O. Neal Miller and Robert E. Olson


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