IN SITU OXIDATION OF LACTATE BY SKELETAL MUSCLE DURING INTERMITTENT EXERCISE

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1. Introduction

At variance with the widely accepted view of Cori and Cori [1] and Himwich et al. [2], a number of data have accumulated indicating that under certain circumstances lactate is oxidized by skeletal muscle [3–9].

Estimation of lactate oxidation at rest and during exercise was made in the isolated perfused gastrocnemius of the dog [10], but the departures from normality associated with the *in vitro* techniques make it doubtful whether conclusions can be drawn for skeletal muscle *in vivo*.

In vivo experiments were carried out in human forearm muscle during exercise by Jorfeldt [11]; however the results obtained in this study afford only an approximate estimate of lactate oxidation.

The present paper is concerned with the *in vivo* oxidation of lactate by dog skeletal muscle, at rest and during intermittent exercise. The investigation was performed under conditions which appear to allow an accurate estimation of the rate of oxidation.

2. Materials and methods

The experiments were performed on the muscles of the lower hind limb of adult dogs anesthetized with pentobarbital. The femoral artery and the femoral vein were exposed just above the knee: all branches to and from the skin and other muscles than those of the lower limb were ligated. Blood clotting was prevented with heparin.

10 mCi L-sodium lactate-U-^{14}C (Radiochemical Centre, Amersham, 0.05 mCi/mmole) were administered into the femoral artery over a 40 sec-period. From the beginning of the tracer infusion the blood flowing through the femoral vein was collected, and measured, in separate fractions. Blood from another dog was transfused into a jugular vein to replace the volume removed. After 2.40–3 min, the collection of blood was stopped and samples of gastrocnemius and tibialis anticus were excised and quickly plunged into liquid N_2. In a number of experiments an infusion of unlabelled lactate (0.15–0.20 M) into the femoral artery, at a rate of approximately 1 ml/10 ml of blood, was started 6 min before the tracer infusion and continued till the end of the experiment.

The investigation was performed in both resting and indirectly stimulated muscles (300 msec trains of supramaximal pulses, 0.5 msec duration, 40/sec, were applied rhythmically every second to the peripheral stump of the sciatic nerve which was sectioned in the thigh). Stimulation was carried out immediately after the end of the tracer infusion.

$^{14}CO_2$ radioactivity of venous blood and tissue was determined by liquid scintillation counting [12]. The values were corrected for the amount of $^{14}CO_2$ present in the commercial preparation of ^{14}C-lactate, as well as for the $^{14}CO_2$ produced by blood metabolism during the circulation time in the limb of the dog (10 sec) [9]. Total non-volatile radioactivity was determined in blood and in muscles by the method of Abraham and Hassid [13]. The fractional (percent) rate of lactate oxidation to CO_2 was calculated relating the mean level of non-volatile radioactivity in muscle to the amount of $^{14}CO_2$ estimated in the muscles at the end of the experiment and in the

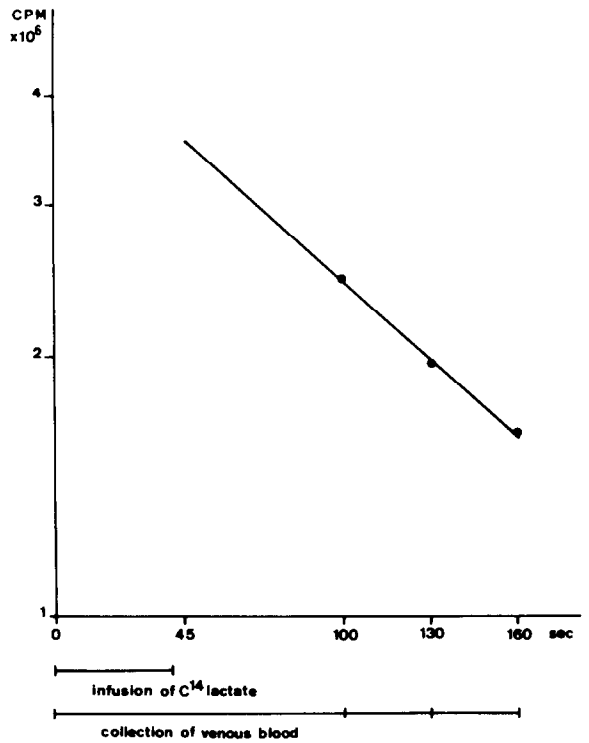

Fig. 1. Non-volatile radioactivity in the muscles of the lower hind limb of a dog following the arterial administration of the label.

blood collected over the experimental period*. It was shown previously that the muscle non-volatile radioactivity decreases after the end of the infusion because of clearance of the label into the blood stream. Direct analysis of muscle samples seemed unsuitable for accurately estimating the maximum tissue content of the label, about 5 sec after the end of the infusion (when the tracer supply to the muscles decreases), because at that time the blood flowing in the muscle vessels was likely to be still carrying a large amount of label which had not diffused into the tissue. On the other hand 1 min after the end of the infusion no significant amount of undiffused label was likely to be trapped in the vascular system. This was confirmed by the results of experiments when colloidal carbon was administered into the femoral artery of the dog: after distributing uniformly in the muscle vessels, the dye was cleared rapidly after stopping the injection, so that no appreciable amount remained after 1 min. It was assumed therefore that the estimation of non-volatile radioactivity in the muscle samples at the end of the experiment (i.e. 2–2.20 min after the end of the infusion) provided an estimate of the amount of the label in the tissue and that any non-volatile ^{14}C which was found in the venous blood later than 1 min after the end of the infusion had diffused back out of the tissue. The change in muscle radioactivity over the last 60 sec was calculated by adding to the final figure determined in muscle by direct estimation the figures determined in the blood fractions collected during the same period: this calculation was checked in two experiments by direct analysis of muscle samples. The values appeared to lie on a straight line when plotted on semilogarithmic paper, conforming to the equation $C_T = C_0 \times 10^{-KT}$ (fig. 1). Extrapolating the line back to 5 sec after the end of the infusion was assumed to afford the figure for the maximum level of tissue non-volatile radioactivity.

Analysis for unlabelled lactate was carried out by an enzymic method [14]. In resting muscles, the lactate content estimated at the end of the experiment was assumed to be equal to the content during the investigation also in the cases when additional unlabelled lactate was administered: under the latter conditions the level was found to remain approximately constant after a 6-min infusion, i.e. during the investigation with the tracer. During rhythmic contractions over 2 min, it was shown by preliminary experiments that the increase in lactate content was linearly related to the duration of exercise. Therefore the average level of lactate during exercise was taken as the arithmetical mean between the final content and the content at rest.

The figures obtained for the fractional rate of oxidation per min and for the level of lactate in muscle over the experimental period allowed the calculation of the amount of lactate oxidized per min/g of muscle.

* Since all non-volatile radioactive compounds other than ^{14}C-lactate were derived from ^{14}C-lactate, identification of non-volatile radio-label seemed unnecessary for the purpose of calculating lactate oxidation to CO_2

3. Results and discussion

The fractional rate of lactate oxidation in the resting muscles was 0.17 ± 0.03% per min. The calculated figure for the amount of lactate oxidized was 2.99 ± 0.63 nmoles/g of muscle per min (table 1) and might reflect merely an exchange in the reversible reaction catalyzed by lactate dehydrogenase.

In the stimulated muscles the fractional rate of lactate oxidation was much increased: 4.98 ± 0.63% per min. The amount of lactate oxidized was 310 ± 68 nmoles/g of muscle per min (table 2).

The fractional rate of oxidation under resting conditions was not increased if the lactate concentration in the muscles was raised by infusion of unlabelled lactate (table 3). It is worth noting that under these conditions the lactate content in the muscles was of the same order of the mean content during stimulation. This shows that the increase of the fractional rate of lactate oxidation during exercise is not mediated by the level of substrate accumulated. The increase in oxidation of lactate is obviously subject to the increase in oxidation of pyruvate. In this respect our results may be correlated with the observations of Linn et al. [15, 16] and of Wieland and Siess [17] that the activity of pyruvate dehydrogenase from kidney, heart and liver is regulated by phosphorylation and dephosphorylation; the enzyme is active in the dephosphorylated form and is inactivated by ATP by way of a phosphorylation reaction. Our data appear to indicate that the same mechanism of regulation may operate *in vivo* in skeletal muscle; the breakdown of ATP following stimulation may be a factor in the activation of pyruvate dehydrogenase.

The increase in lactate production during exercise can be explained, in accordance with Keul et al. [18] and Jöbsis and Stainsby [19], by assuming that in the exercising muscle the increase in pyruvate formation exceeds the increase in pyruvate oxidation; lactate accumulates because more pyruvate is formed than can be oxidized in the mitochondria. The oxidative removal of lactate in the rhythmically exercising mus-

Table 1
Oxidation of lactate in skeletal muscle at rest without addition of unlabelled lactate.

Exp. no.	Fractional rate of lactate oxidation, (% per min)	Lactate content in muscle, (μmoles/g)*	Lactate oxidized, (nmoles/g per min)
5	0.27	1.8	4.86
7	0.15	1.7	2.55
27	0.14	1.5	2.10
28	0.13	1.9	2.47
Mean ± S.E.	0.17±0.03	1.7±0.09	2.99±0.63

* Values were estimated at the end of the experiment.

Table 2
Oxidation of lactate in skeletal muscle during rhythmic exercise.

Exp. no.	Fractional rate of lactate oxidation, (% per min)	Mean lactate content in muscle (μmoles/g)	Lactate oxidized (nmoles/g per min)
6	6.35	4.5	286
8	4.02	3.8	153
24	3.81	8.3	316
25	5.76	8.4	484
Mean ± S.E.	4.98±0.63	6.2±1.21	310±68

Table 3
Oxidation of lactate in skeletal muscle at rest with an added infusion of unlabelled lactate.

Exp. no.	Fractional rate of lactate oxidation, (% per min)	Lactate content in muscle (μmoles/g)*	Lactate oxidized (nmoles/g per min)
11	0.18	2.7	4.86
19	0.28	4.7	13.16
20	0.05	3.2	1.60
22	0.08	7.9	6.32
Mean ± S.E.	0.15±0.05	4.6±1.17	6.48±2.43

* Values were estimated at the end of the experiment.

cle probably occurs during the resting periods, because the imbalance between the rates of pyruvate production and pyruvate oxidation is reversed. It is suggested that the imbalance is reversed because the acceleration of pyruvate oxidation outlasts the acceleration of glycolysis after each train of stimuli.

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