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Lactate Oxidation by Three Segments of the Rabbit Proximal Tubule (42365)

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Abstract. Oxidation of [U^{14}C]lactate to $^{14}CO_2$ was measured *in vitro,* in nonperfused anatomically defined segments of rabbit proximal tubule (S1, proximal convoluted, and S2 and S3, proximal straight tubules). The rate of lactate oxidation was similar in S2 and S3 segments, and within the range of lactate oxidation rates measured *in vivo.* In contrast, the oxidation rate of S1 segments was significantly lower than that of S2 or S3. In proximal straight tubules, lactate oxidation was inhibited by incubation at 0°C, or by application of 1 m*M* ouabain. To determine if the rate of transepithelial transport affected the rate of lactate oxidation, lactate oxidation was measured in proximal straight tubules after the lumen had been opened by perfusion with Ringer's containing 10 m*M* polyethylene glycol. No difference in lactate oxidation rate was observed between tubules with patent lumina and nonperfused tubules. These results suggest that the various segments of the renal proximal tubule have different metabolic characteristics, and that the rate of substrate oxidation is related to the activity of the Na^+, K^+-ATPase.

The role of lactate oxidation in providing energy for the kidney has been examined both *in vivo* (1, 2) and *in vitro* (3, 4). Dies *et al.* (2) have provided evidence that lactate oxidation supports renal sodium reabsorption in the dog. Brand *et al.* (1) reported that lactate is oxidized at a substantial rate by the dog kidney *in vivo,* although in this study, no relationship was found between lactate oxidation and sodium reabsorption. However, Klein *et al.* (4) have presented data, based on observations with individual nephron segments, that lactate is not oxidized by the proximal tubule of the rat. Since the proximal tubule is responsible for the largest fraction of net sodium reabsorption, the results reported by Dies *et al.* (2) and Klein *et al.* (4) seem partially contradictory. In view of these different findings, we reexamined the question of lactate decarboxylation by the proximal tubule.

We report here experiments measuring the rate of lactate oxidation in the three histologically distinct segments of the rabbit proximal tubule, the S1, S2, and S3 segments (5). We confirmed Klein's observation that the rate of lactate oxidation in the early proximal convoluted segment (S1) is very low. However, in the S2 and S3 segments, the rate of lactate oxidation is higher and similar to the rate reported for the kidney *in vivo.*

Methods. *Dissection of tubules.* New Zealand white rabbits of either sex were killed by a blow to the occiput. The kidneys were removed, decapsulated, and sliced with a Stadie–Riggs microtome. The slices were placed in a phosphate-buffered Ringer's solution (composition given below) containing 0.1 g/100 ml of dialyzed, defatted bovine serum albumin.

For dissection of outer cortical proximal convoluted tubules, the kidney was sliced tangentially, the first slice (largely cortex corticis) discarded, and the tubules were dissected from the second slice.

Tubules of S1 type were obtained by selecting only segments with adherent glomeruli. The glomeruli were discarded before incubation. Cortical to midcortical proximal straight tubules of predominantly S2 cell type were dissected from transverse slices, utilizing tubule segments which coursed from the cortical surface toward the corticomedullary junction. Tubules of S3 type were dissected from the outer medulla and identified by their transition into the beginning of the thin descending limb.

As they were dissected, the tubule segments were transferred to a metabolic incubation chamber which was kept on crushed ice. (The plexiglass incubation chamber is similar to the one described by Le Bouffant *et al.* (6), except that we did not use dry collagen.) When a sufficient number of tubules had been obtained for an experiment (usually four tubules per chamber), the size of each tubule in the chamber was measured with a Bausch and Lomb

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projecting microscope. Tubular volume was calculated assuming that the tubules were simple cylinders. The highly convoluted S1 segments were cut into short pieces (0.3 mm) to facilitate accurate drawing of their image. Metabolic rate was factored by the total volume of tubules in each chamber.

Incubation of tubules. After the tubules were measured, the rate of oxidation of lactate to CO_2 was measured. The albumin-containing Ringer's was replaced with a bath solution consisting of 10 μl of colloid-free, phosphate-buffered Ringer's containing 1.28 m*M* (L)(+)-[U^{14}C]lactate (25 μCu/ml) (ICN, Irvine, Calif.), pH 7.4. Four aliquots of the bath were taken with a precisely calibrated nanoliter pipet (70–80 nl) for radioassay of actual bath ^{14}C concentration. Then the surface of the Ringer's solution was covered with water-saturated, water-washed decane to prevent evaporation. The incubation chamber lip was coated with petroleum jelly and sealed with a cover slip. On the bottom of the coverslip (i.e., inside the chamber airspace) a fragment of another cover slip was fastened with petroleum jelly. From this fragment a 0.2-ml droplet of 0.3 *M* KOH was suspended. The incubation chamber was placed in a water bath at 38°C.

At the end of 30 min of incubation, the chamber was removed from the water bath and the glass fragment with the KOH droplet recovered for radioassay of any $^{14}CO_2$ trapped in the KOH. For subsequent incubation with the same tubules, the chamber was resealed with a fresh KOH droplet and returned to the 38°C water bath. In most experiments, three such consecutive 30-min incubation periods were obtained with the same tubules.

To determine the extent to which $^{14}CO_2$ was liberated from the bath due to bacterial action or spontaneous decarboxylation, in each experiment one incubation chamber was carried through all the incubation procedures as just described, except that tubules were omitted. The radioactivity in the KOH droplets recovered from this chamber (blank value) was subtracted from the radioactivity in the KOH samples of chambers with tubules. The count rate in the blanks averaged 7.8% of the count rate in the experimental samples.

Maintenance of patent lumen. In one series of experiments, the effect of maintaining patency of the tubular lumen on lactate oxidation rate was measured. Proximal straight tubules were dissected as usual and transferred to a perfusion dish. Under a stereomicroscope, the tubules were perfused with Ringer's containing 10 m*M* polyethylene glycol (PEG) (mol wt 8000; Sigma Chemical Co.). In order to prevent diffusion of PEG out of the cut ends of the tubules, PEG was added to the bath at 10 m*M* throughout the remainder of this experiment. The method of tubule perfusion is based on that originally reported by Burg *et al.* (7) and used previously by us (8). Opening of the tubular lumen during perfusion is readily observed with the stereomicroscope.

After the lumina were opened, the tubules were detached from the perfusion pipets and transferred to the metabolic incubation chamber. Lactate oxidation to CO_2 was determined as usual. At the end of the three 30-min incubation periods, patency of the lumen was confirmed in all tubules perfused with PEG, by examination with an inverted phase contrast microscope at 300×.

To obtain longer tubules so as to facilitate perfusion, combined S2 and S3 segments were used. In each experiment, two groups of four tubules were used. After dissection, all the tubules were drawn in the collapsed state. Then the four experimental tubules were perfused with PEG as above and transferred to the metabolic chamber. The incubation bath for the other (control, nonperfused) tubules also contained 10 m*M* PEG, to test for any direct effect of PEG on lactate oxidation.

Solutions. The phosphate-buffered Ringer's contained (m*M*): NaCl, 130; KCl, 5; NaH_2PO_4, 4; $MgSO_4$, 1; $CaCl_2$, 1.8; and Na lactate, 1.28. The Ringer's was titrated to pH 7.4; final [Na^+] was 135 m*M* and osmolality, 286 mOsm/kg. The Ringer's solution was equilibrated with room air for oxygenation. A calculation assuming a rate of oxygen consumption at the high end of the normal range for kidney tissue indicated that during 30 min of incubation within a sealed chamber, the tubules would consume only 2% of the dissolved oxygen. Consequently, there was no need to increase the PO_2 of the Ringer's above ambient.

Radioassay. The quadruplicate aliquots of bath and the glass fragments with KOH were transferred to 8-ml scintillation minivials containing 0.1 ml water. Six milliliters of a

scintillation cocktail (8) was added and the samples were assayed for ^{14}C activity in a Packard Model 4530 liquid scintillation spectrometer. Samples were counted to 1% standard deviation of counting (10,000 counts).

Calculations. The lactate oxidation rate, picomoles lactate oxidized to CO_2 per nanoliter tubule per min, was calculated as follows:

$$\text{lactate oxidation rate} = (\text{cpm KOH} - \text{cpm blank})/(SA_b)(V)(t),$$

where (cpm KOH − cpm blank) represents the net radioactivity due to $^{14}CO_2$ trapped in the KOH, SA_b is the bath lactate specific activity (cpm/picomoles), V is the total volume of tubules in the chamber (nliters), and t is the incubation time (min).

Statistics. Data are expressed as the means ± one standard error. Results were analyzed by a paired Student t test where possible, or by an unpaired t test otherwise. Statistical comparisons were made only to test null hypotheses posited before performance of the experiments, in order to avoid generating false positive results by use of repeated t tests on the same data (9).

Results. *Recovery.* In order to determine if CO_2 produced by the tubules could be quantitatively trapped in the KOH droplet, $^{14}CO_2$ recovery experiments were performed. In six experiments, a droplet of $NaH^{14}CO_3$ solution was added to an incubation chamber (without tubules or [^{14}C]lactate). After sealing the chamber, $^{14}CO_2$ was liberated from $NaH^{14}CO_3$ by mixing the $NaH^{14}CO_3$ solution with a droplet of an acid phosphate buffer (pH 5.5) already present, using a magnetic flea. The amount of ^{14}C activity added as $NaH^{14}CO_3$ was similar to the amount of $^{14}CO_2$ anticipated in experimental samples. Recovery of $^{14}CO_2$ activity in KOH was 96.2 ± 0.37%. These data show essentially complete recovery of CO_2 in the KOH droplet.

The possibility remains that during the experimental incubations, some of the $^{14}CO_2$ generated from metabolism of [^{14}C]lactate is not released from the incubation medium and so not trapped in the KOH droplet. To the extent that $^{14}CO_2$ is not released from the medium, the measured rate of conversion of lactate to CO_2 will be underestimated. The extent of this underestimation is probably minimal in these experiments for two reasons. First, the measured rates of lactate oxidation are similar to those reported by others (see below). Second, the KOH droplet in the air space of the incubation chamber acts as a sink for CO_2, necessarily maintaining a very low PCO_2 in the atmosphere of the chamber, and thereby providing a driving gradient for release of metabolically produced CO_2 from the medium to the KOH droplet.

Volume per unit length of tubules. Our data are expressed per unit tubule volume, to allow comparison of oxidation rates for segments with different diameters. To compare our oxidation rates to those found by others, in our experiments the volume per unit length for the tubule segments was: S1 segments, 1.46 $nl \cdot mm^{-1}$; S2 and S3 segments, 1.01 $nl \cdot mm^{-1}$.

Effect of incubation time. To examine the effect of time on lactate oxidation rate, using S3 segments, the rates for the first, second, and third consecutive 30-min incubation periods for control tubules in 10 experiments (40 tubules from 10 rabbits) were compared. The absolute oxidation rates were similar to those in other experiments (see below) but there was considerable variation in rates among tubules from different rabbits. Therefore, the results were expressed for each experiment (animal) as a percentage of the oxidation rate for the first 30-min period (=100%). For the second 30-min period, the mean oxidation rate was 104%, and for the third, 85%. At most, the rate of lactate oxidation to CO_2 decreases by 15% over 90 min, an amount too small to affect the conclusions drawn from these experiments.

Comparison of oxidation rates of S1, S2 and S3 segments. To determine if lactate oxidation rates vary in different parts of the proximal tubule, the rate in S3 segments was compared to the rate in S1 and S2 segments.

In five experiments, the lactate oxidation rate in outer cortical PCT (S1) segments was compared to that in S3 segments. In each experiment, the oxidation rate in four S3 segments was compared to the rate in 9–12 S1 segments taken from the same rabbit. The S1 segments had a mean diameter of 43 μm, while the S3 segments had a diameter of 32 μm. The total volume of tissue present in the S1 incubation chambers was 5.91 ± 1.13 nl per chamber, while in the S3 chambers, 2.88 ± 0.25 nl was present.

The lactate oxidation rates for five experiments comparing S1 and S3 segments, and for five other experiments, comparing S2 and S3 segments, are shown in Table I. As seen in the table, the oxidation rate in S1 segments was very low, close to the limit of measurement of this method and significantly lower than the oxidation rate of S3 segments of tubules from the same rabbits. In contrast, there was no significant difference in the oxidation rate of S2 and S3 segments. Thus the rate of oxidation of lactate to CO_2 is similar in the two segments of the proximal straight tubule, and fivefold greater than the rate in the initial proximal convoluted tubule.

Effect of ouabain and of low temperature on lactate oxidation. To evaluate the extent to which lactate oxidation was coupled to ion transport, the effect of ouabain on lactate oxidation was measured. In this series of experiments, we also tested the effect of incubation at 0°C to further examine the validity of our measurement of substrate oxidation.

Six experiments were performed, using two groups of four S3 tubules in each. In one group of tubules, the first and third 30-min periods served as controls (38°C) while the second incubation period was used to test the effect of low temperature by placing the chamber on a slurry of crushed ice for 30 min. A preliminary study showed that the temperature in the incubation chamber decreased to 0°C within 6 min after being placed on ice. Therefore, a preliminary 10-min equilibration period on ice was allowed, and then the lactate oxidation rate at 0°C was measured during the next 30 min.

There was no difference in the lactate oxidation rate between the first and third periods in the control group (38°C) and so these data were pooled. The results are shown in Table II. Incubation at low temperature significantly decreased the oxidation rate by 93%, an effect which was completely reversible.

TABLE I. LACTATE OXIDATION TO CO_2 IN S1, S2, AND S3 SEGMENTS[a]

	pmol Lactate · min^{-1} · nl^{-1}	P
S1	0.048 ± 0.023[b]	
S3	0.235 ± 0.069	$p < 0.025$
S2	0.312 ± 0.051	
S3	0.485 ± 0.142	$p > 0.2$

[a] $N = 5$.
[b] Mean ± standard error.

TABLE II. LACTATE OXIDATION TO CO_2: COLD, OUABAIN, PEG

	pmol Lactate · min^{-1} · nl^{-1}	P
Control 38°C	0.182 ± 0.026	
1 m*M* ouabain	0.076 ± 0.019	$p < 0.05$
0°C	0.013 ± 0.003	$p < 0.001$
Control	0.175 ± 0.016	
PEG perfused	0.155 ± 0.030	NS[a]

[a] NS, not significant.

Ouabain, 1 m*M*, was added to the bath for incubation of the second group of tubules. The tubules were exposed to ouabain during three consecutive 30-min incubation periods. Separate blanks were run for the control and ouabain-containing baths. Incubation with ouabain lowered the lactate oxidation rate in all three periods (Table II). Ouabain inhibited lactate oxidation by 58%, suggesting that more than half of the lactate oxidized is supporting active Na^+, K^+-ATPase-dependent ion transport.

Effect of luminal patency. Lactate oxidation may have been depressed if collapse of the lumen in nonperfused tubules limited the rate of transepithelial active Na^+ transport. To determine if such a limitation occurs, the rate of lactate oxidation was compared in nonperfused tubules versus tubules with patent lumina.

Five experiments measuring lactate oxidation in patent (S2 + S3) tubules were attempted. The control tubules were accidentally lost in one. The mean length and volume (before perfusion) of the perfused tubules were 1.29 ± 0.085 mm and 1.09 ± 0.135 nl, respectively. For control (nonperfused) tubules, the length = 1.03 ± 0.092 mm, and volume = 0.790 ± 0.08 nl. The lactate oxidation rate was not significantly different between the two groups (Table II). (The data for 30, 60, and 90 min are pooled and the mean for all three periods given.) Maintaining luminal patency did not increase the rate of lactate oxidation in S2 + S3 segments. Possible reasons for the lack of any effect of luminal patency are given in the discussion.

Discussion. *Validity of the method.* The measured rate of lactate decarboxylation represents tubular cell metabolism:

(a) The rate of volatile ^{14}C trapping in KOH in the absence of tubules (blanks) is only 8% of the rate in the chambers containing tubules, indicating that neither bacterial action nor spontaneous decarboxylation of [^{14}C]lactate introduce a significant error.

(b) The rate of decarboxylation decreases by no more than 15% during the 90-min incubation period.

(c) Decreasing temperature from 38 to 0°C decreases metabolic rate by 93%, and rewarming to 38°C results in complete recovery. This reversible depression in $^{14}CO_2$ production with low temperature is characteristic of metabolically active tissue.

(d) The rate of lactate decarboxylation in these isolated tubules is similar to the rate of lactate oxidation by mammalian kidney under other conditions. Assuming that the density of kidney tissue is 1.00, the units of (pmole $min^{-1} \cdot nl^{-1}$) are equivalent to (μmole g^{-1} min^{-1}). Thus the rate of decarboxylation in S2 and S3 segments is equivalent to 0.18–0.49 μmole min^{-1} g^{-1} (Tables I and II). The rate of lactate conversion to CO_2 by dog kidney *in vivo* has been reported as 0.28 (1) to 0.40 (2) μmole min^{-1} g^{-1}. For isolated, perfused rat kidney, the rate is 0.6–0.7 μmole min^{-1} g^{-1} (3). Although the rate of lactate oxidation in the intact kidney may reflect the activity of more distal segments as well as the proximal tubule, and the flow of blood and urine may affect the rate of substrate utilization, the similarity of the rate observed in isolated tubules to the rate *in vivo* suggests that these segments effect a significant proportion of renal lactate oxidation *in vivo.* In addition, this similarity of lactate decarboxylation rates measured *in vivo* and in isolated tubules supports the validity of our method of measurement of tubular lactate oxidation.

The method for measuring lactate oxidation to CO_2 reported here is similar to that developed by Le Bouffant *et al.* (10). Klein *et al.* have used a somewhat different method for measurements of substrate oxidation to CO_2 in individual nephron segments (4).

Low oxidation rate in S1. Our finding of a low rate of lactate oxidation in S1 segments confirms Klein *et al.*'s observation (4). However, we have now shown that there is a substantial rate of lactate oxidation in S2 and S3 segments.

In contrast to the very low rate of lactate oxidation in S1 segments that we report here, Le Bouffant *et al.* (10) observed that the rat proximal convoluted tubule produced CO_2 from lactate at a rate of 1.37 pmole $CO_2 \cdot min^{-1}$, $\cdot mm^{-1}$. Recalculating their results according to our method yields an oxidation rate of 0.46 pmole lactate, $min^{-1} \cdot mm^{-1}$. This rate is comparable to the rate we found for S2 and S3 segments but much higher than the rate for proximal convoluted segments (Table I). A possible explanation for the apparently higher lactate oxidation rate in PCT in Le Bouffant *et al.*'s study may relate to the tubule segment actually used. The segment illustrated by Le Bouffant *et al.* (6) as a proximal convoluted tubule more closely resembles the later, relatively straight segments of the proximal tubule, and not the highly convoluted initial segment used in our studies. Thus the oxidation rate reported for PCT by Le Bouffant *et al.* may, in fact, represent an oxidation rate for the S2 or S3 segment.

Two possible explanations for the low rate of lactate oxidation in the S1 segment are that lactate might not gain entry to the cell in this segment, or that the S1 segment might lack appropriate enzymes for lactate oxidation.

The low rate of lactate oxidation in the S1 segment is probably not due to inability of lactate to enter the tubular cell. There are specific transport pathways for lactate in both the luminal and basolateral membrane of the proximal tubule in rat and rabbit kidney (11, 12). In the rat kidney (13) lactate disappears from the tubular lumen under free-flow conditions in the earliest proximal convolutions, which approximately correspond to the S1 segment. These results suggest that lactate has access to the tubular cell from either membrane in all segments of the proximal tubule. Failure of lactate to gain entry to cells in the S1 segment is an unlikely explanation for the low oxidation rate.

The different lactate oxidation rates in S1, S2, and S3 segments reported here are similar to the distribution of lactate dehydrogenase activity in the rabbit proximal tubule. Endou

et al. (14) reported that lactate dehydrogenase activity is lowest in the S1 segment of the rabbit proximal tubule, and higher in the S2 and S3 segments. Lactate enters the metabolic oxidative pathway via oxidation to pyruvate, a step catalyzed by lactate dehydrogenase. The low activity of this enzyme in the S1 segment may explain the low rate of lactate oxidation to CO_2. Alternatively, the lower oxidation rate for the S1 segment might reflect a greater sensitivity of this segment to the *in vitro* conditions used in our study.

Luminal patency and lactate oxidation. If lactate oxidation to CO_2 were dependent on the rate of transepithelial inorganic ion transport, then increasing the rate of transport should increase the rate of lactate oxidation. Accordingly, an increase in lactate oxidation was expected when lactate oxidation in tubules with patent lumina were compared to nonperfused tubules.

The failure to observe an increase in lactate oxidation in the tubules perfused with PEG means either that the maximal rate of transepithelial ion transport was already occurring, or that lactate oxidation is not coupled to ion transport.

The latter explanation seems less likely in view of the 58% inhibition in lactate oxidation brought about by ouabain. However, the effect of ouabain on lactate oxidation could be indirect, and mediated via changes in intracellular milieu secondary to inhibition of ion transport. Against this possibility is recent evidence providing further support for a central role of the Na^+, K^+-ATPase in coupling of mitochondrial oxidation to Na^+ and K^+ transport in the proximal tubule (15). An effect of ouabain to inhibit ion transport and thereby decrease mitochondrial oxidation is compatible with this hypothesis and provides the simplest explanation for the observed effect of ouabain on lactate oxidation in our experiments. Why, then, is there no effect on lactate oxidation when the lumen is opened?

One possibility is that in the nonperfused tubule, the lumen is in fact accessible to the bath. If conditions were such that the rate of lumen to bath ion and fluid reabsorption were low, then backleak of ions and water via the paracellular channels, and leakage into the lumen at the cut end of the tubules, might provide a sufficient source for continued recycling of ions and water despite apparent collapse of the lumen. Under these conditions, making the lumen patent would not result in any increase in ion transport and transport-dependent catabolism.

The relatively low rate of fluid and ion reabsorption in the proximal straight tubule, used in these experiments, compared to the proximal convoluted tubule, increases the likelihood that sufficient backleak could occur to support fluid recycling in the nonperfused tubule. Also, in our experiments, the absence of reabsorbable organic solutes other than lactate would decrease by 50% the rate of fluid reabsorption by rabbit proximal straight tubules (16).

If this explanation for the lack of effect of perfusion with PEG is correct, it implies that the lumina of nonperfused tubules is readily accessible to the bath, and that under these conditions significant continued fluid and ion reabsorption occurs. Consequently, caution must be exercised in interpreting nonperfused tubule experiments with regard to the assumption that transport or metabolism may be primarily limited by basolateral membrane events. Arthus *et al.* (17) have presented evidence for some degree of accessibility of the tubular lumen in nonperfused tubules.

In conclusion, lactate is oxidized to CO_2 by S2 and S3 segments of the rabbit proximal tubule at a rate comparable to the rate of lactate oxidation by the whole kidney *in vivo*. In these segments, lactate oxidation is probably coupled to active Na^+ and K^+ transport. The low rate of lactate oxidation observed in the S1 segment may be explained by the low activity of lactate dehydrogenase in this segment.

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