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D- and L-Lactate Catabolism to CO_2 in Rat Tissues[1,2] (41803)

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Abstract. The current study was initiated in order to compare the rates of oxidative catabolism of D- and L-lactate in various rat tissues. Uniformly labeled D- or L-[^{14}C]lactate was incubated at 37°C in a closed system with tissue homogenates in Krebs–Ringer phosphate buffer. Evolved $^{14}CO_2$ was trapped in a center well containing a fluted filter paper saturated with strong base and the radioactivity determined. The ratio of L-lactate to D-lactate oxidation was greatest in brain, followed by kidney, heart, and liver. In liver the rate of oxidation of D-lactate exceeded that of L-lactate, in heart the rates were not significantly different and in the other two tissues L-lactate was oxidized more rapidly than D-lactate. These results indicate that the rate of D-lactate catabolism is considerable and is relatively greater than had been reported previously.

By the early 1900s it was clear that the predominant form of lactic acid in mammals was the L-(+) isomer (1). However, the "unnatural" isomer, D-(−)-lactate, was reported (2, 3) to be synthesized in homogenates of mammalian tissues from added methylglyoxal (MeG). This catalysis is now known to occur through a dual-enzyme system, glyoxalase I (*S*-lactoyl-glutathione methylglyoxal-lyase, isomerizing; EC 4.4.1.5) and glyoxalase II (*S*-2-hydroxyacylglutathione hydrolase; EC 3.1.2.6) (4) with glutathione (5) as a coenzyme. The formation of D-lactate has been shown in adult human red blood cells under conditions where glycolysis was inhibited or dihydroxyacetone phosphate was added (6, 7). The low (0.02 m*M*) but consistent plasma level of D-lactate in adult humans (7) and rats (8) is suggestive of metabolic control.

Some investigators have reported a small capacity for utilization of D-lactate compared to that of the L form (9–11), while others (12–15) presented contradictory data. The model systems used included whole body, slices, organ perfusion, and homogenates.

The involvement of D-lactate in human genetic abnormalities (16) and neurological disturbances in both adults (17, 18) and children (19) suggest that D-lactate has a physiological importance beyond that previously expected. Examples include: D-lactate aciduria in children with genetic diseases (16) and in premature infants fed or infused with D-lactate (20), D-lactate acidosis with neurological disturbances in adults (17, 18) and children (19) following bowel resection with subsequent overgrowth of bacteria that synthesizes D-lactate.

The inconsistency of literature reports on D-lactate catabolism in tissues as well as the potential importance of D-lactate in humans have led us to attempt to determine the relative rates of D- and L-lactate catabolism to CO_2 for a wide range of tissue homogenates.

Materials and Methods. Catabolism of uniformly labeled D- or L-[^{14}C]lactic acid to $^{14}CO_2$ was measured in 25 ml rubber-stoppered (Kontes K-882310) Erlenmeyer flasks with plastic center wells (Kontes K-882320) for $^{14}CO_2$ collection. All incubations were in Krebs–Ringer phosphate buffer (KRP) at pH 7.4, similar to that described by Piontek *et al.* (21). The KRP was filtered through a filter (Millipore) of 0.45-μm pore size, stored in glass at 4°C, and oxygenated with 95% O_2–5% CO_2 for 3–5 min prior to use. Volumes of homogenates containing from 6 to 360 mg of tissue were added to the flasks. The amounts of tissue used were determined from the data in Figs. 1 and 2. KRP was added to give a final volume of 5.0 ml. The flasks with tissue preparations, KRP, and unlabeled substrate

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were preincubated for 5 min at 37°C in a Dubnoff shaker bath. The stopper with a center well that contained a 13 × 50-mm flutted Whatman No. 1 filter paper and 0.1 ml hyamine hydroxide (New England Nuclear) was added after addition of 10 μl of 50 μCi/ml radioactive substrate to the reaction mixture. Flasks were incubated in the shaker bath at 37°C for various time periods, from 10 to 60 min. The reactions were terminated by injection of 1.0 ml of 2 *N* perchloric acid (PCA) through the stopper, and flasks were incubated for 1 hr at 37°C to trap the evolved $^{14}CO_2$ on the filter paper. The filter paper was added to scintillation vials, and the center well rinsed with scintillation fluid. The scintillation fluid was 4 g PPO and 50 mg POPOP per liter of toluene. Radioactivity was measured in a Beckman scintillation counter.

For zero-time flasks, PCA was added to the tissue in the reaction flask prior to the addition of the radioactive substrate. The flasks were capped and incubated 1 hr to trap evolved $^{14}CO_2$. Flasks containing radioactive substrate, but no tissue, were also used as controls. For flasks receiving supplemental cold D-lactate (only those for D-[^{14}C]lactate oxidation), 18 m*M* D-lactate in KRP was added; the actual amount depended upon the tissue used and was based on the amount of D- and L-lactate present in the tissue. L-Lactate determination showed a significant concentration of this isomer. The concentration of L-lactate in various tissue homogenates was determined for 2-day fasted rats (Table I). For measurement of $^{14}CO_2$ from D-lactate, tissues were supplemented with unlabeled D-lactate at the levels

TABLE I. L-LACTATE CONTENT OF TISSUE HOMOGENATES FROM 2-DAY FASTED RATS

Tissue[a]	L-Lactate[b] ± SEM (*N*)
Kidney	8.4 ± 0.3 (2)
Heart	31.8 ± 0.1 (2)
Liver	3.4 ± 0.4 (12)
Brain	12.0 ± 0.2 (2)

[a] Homogenates containing 0.1 g tissue · ml^{-1} of homogenate were used for determinations. Homogenates were prepared as described under Methods except that all tissues were homogenized additionally for 30 sec with a Tekmar Tissuemizer before determination of L-lactate.

[b] All values were expressed as μmole L-lactate · g^{-1} tissue (wet weight).

TABLE II. D-[^{14}C]LACTATE CATABOLISM AS A FUNCTION OF THE ADDITION OF UNLABELED D-LACTATE TO LIVER HOMOGENATES[a]

Unlabeled D-lactate[b]	cpm ± SEM (*N*)
0	9016 ± 1269 (3)
3.4	8808 ± 1301 (3)
6.8	5171 ± 604 (3)
13.6	2723 ± 643 (3)
27.2	1160 ± 171 (3)

[a] All flasks contained 10 μl D-[U-^{14}C]lactate and 300 mg liver in 5 ml of incubation solution; incubated 20 min at 37°C and $^{14}CO_2$ measured as described under Methods.

[b] μmole · g^{-1} tissue (wet weight).

of 8.4, 32, 3.4, and 12 μmole · g^{-1} tissue for kidney, heart, liver, and brain, respectively. Determination of D-lactate in tissue homogenates showed concentrations less than 0.005 μmole/g tissue. Thus, total D- and L-lactate content in the incubation mixtures were approximately the same. The amount of unlabeled D-lactate did affect $^{14}CO_2$ evolution from D-[^{14}C]lactate as shown in Table II for liver homogenates. D-lactate was determined by the specific enzymatic method of Brandt *et al.* (7) and L-lactate was determined with a Sigma assay kit 826-UV.

Radioactive substrates. Uniformly labeled D- and L-[^{14}C]lactate from ICN, California, was diluted to 50 μCi/ml and the pH adjusted to 3–4 with 0.12 *N* HCl. N_2 gas was bubbled through the substrate solution to remove volatile impurities which were found to cause a high background. The purity of the D- and L-lactate was determined by paper chromatography of D- and L-lactate dehydrogenase (LDH)-treated samples. D-[^{14}C]lactic acid was added to glycine–hydrazine-buffer, pH 9.5 (7), and triplicate samples were quantitatively added to Whatman No. 1 paper in separate lanes. The remaining solution was incubated with L-LDH (L-lactate: NAD^+ oxidoreductase; 1.1.1.27) for 0.5 hr at 37°C and triplicate aliquots added to separate lanes. This solution was incubated with D-LDH from *Lactobacillus leichmanni* (D-lactate: NAD^+ oxidoreductase; 1.1.1.28) for 1.5 hr at 25°C and triplicate aliquots added to separate lanes. Ascending chromatography in a saturated tank for 3 hr using *n*-butanol–acetic acid–H_2O (12:3:5) (22) was followed by air drying. One-centimeter strips were cut and added to scintillation tubes.

By measuring the radioactivity for each experimental protocol the maximum L-lactate contamination in the D-lactate labeled compound was shown to be 5%. Similar chromatography for L-[^{14}C]lactic acid after treatment with D-LDH, followed by L-LDH showed maximum contamination of 3%.

Tissue preparations. Male Sprague–Dawley rats from 295 to 457 g were fasted for 2 days, decapitated, and blood collected into 103 × 29-mm polypropylene centrifuge tubes containing 100 USP units of sodium heparin. The tissues were rapidly removed, rinsed in saline, and placed in plastic dishes on ice. For liver preparations, after the rat was decapitated, the azygous vein was cannulated and cold saline was used to perfuse the liver under gravity flow with the blood and saline exiting through the cut posterior vena cava. Other tissues were partially perfused by this technique. After 10–15 min, the liver was removed, washed with saline, and placed in a plastic dish on ice. Cold homogenates, using 9 or 19 vol of KRP/g of tissue, were prepared with a Teflon pestle.

Statistical analysis. Statistical analyses (mean, SEM, linear regression, and linear correlation) were performed using a Texas Instrument-59 programmable calculator. P values were determined from a two-tailed Student's t test and from tables of significance limits for r values from the sample correlation coefficient.

Results. *Effect of tissue concentration on $^{14}CO_2$ formation from D- or L-lactate.* Figure 1 shows the plot of $^{14}CO_2$ formed from D- or L-lactate expressed in counts per minute (cpm) as a function of the amount of tissue from kidney, heart, liver, and brain homogenates. The cpm · g^{-1} of tissue · min^{-1} may be determined from the linear regression equations from the data. The largest tissue amounts were not included in the linear regression analyses. The r values indicate significance at least at the $P < 0.05$ for all the tissues and isomers. A tissue-amount dependency for the catabolism of both D- and L-lactate was shown. These data were used to determine a tissue amount where the reactions were linear with the tissue added. These experiments allowed the determination of a time course of reaction (Fig. 2) for a comparison of the relative rates of oxidation of D- and L-lactate.

Time course for $^{14}CO_2$ formation from D- or L-lactate. Figure 2 shows the plots of $^{14}CO_2$ formed from D- or L-lactate, expressed in cpm as a function of time from 0–30 min. The tissue amounts were chosen from the linear portion of the curves shown in Fig. 1. These experiments were performed to compare the rates of D- and L-lactate catabolism under the same conditions of tissue amount, isomer concentration, incubation time, and other experimental conditions. The cpm · g^{-1} of tissue · min^{-1}, may be determined from the linear regression equations from the data of Fig. 2. The r values indicate significance of at least $P < 0.05$ for all tissues and both isomers from 0–30 min. The cpm · g^{-1} of tissue · min^{-1} for each tissue for specific tissue weight and 20-min incubations are shown in Fig. 3. These data show that L-lactate catabolism was significantly different from D-lactate catabolism at least at the $P < 0.01$ level in kidney, liver, and brain homogenates. Catabolism of the two isomers in heart were not significantly different. For D-lactate, only catabolism in liver compared to brain, and kidney compared to heart were not significantly different. All of the other tissue comparisons for the D- isomer were significantly different at the $P < 0.001$ level. The catabolism of L-lactate to $^{14}CO_2$ was significantly different in all tissues at least at $P < 0.005$.

$^{14}CO_2$ Formation in other tissue homogenates. The results for formation of $^{14}CO_2$ from D- and L-lactate from five other tissue homogenates are shown in Fig. 4. These tissues have not been as extensively studied, but none of them have D-lactate catabolism as large as that shown in Fig. 3 for kidney, heart, liver, and brain. Unlabeled D-lactate was not added. If the D oxidation is adjusted for the L-isomer isotopic contaminant, the D-isomer oxidation is nearly absent in comparison to that of L-, with the exception of small intestine, where both isomers are poorly oxidized. Ratios are not reported due to the preliminary nature of this data.

Discussion. Initial experiments were carried out with liver slices. Similar but not identical results were obtained with liver homogenates. D-[^{14}C]Lactate oxidation was about the same with both tissue preparations while L-[^{14}C]lactate oxidation was less with homogenates than with slices. The ratio of oxidation

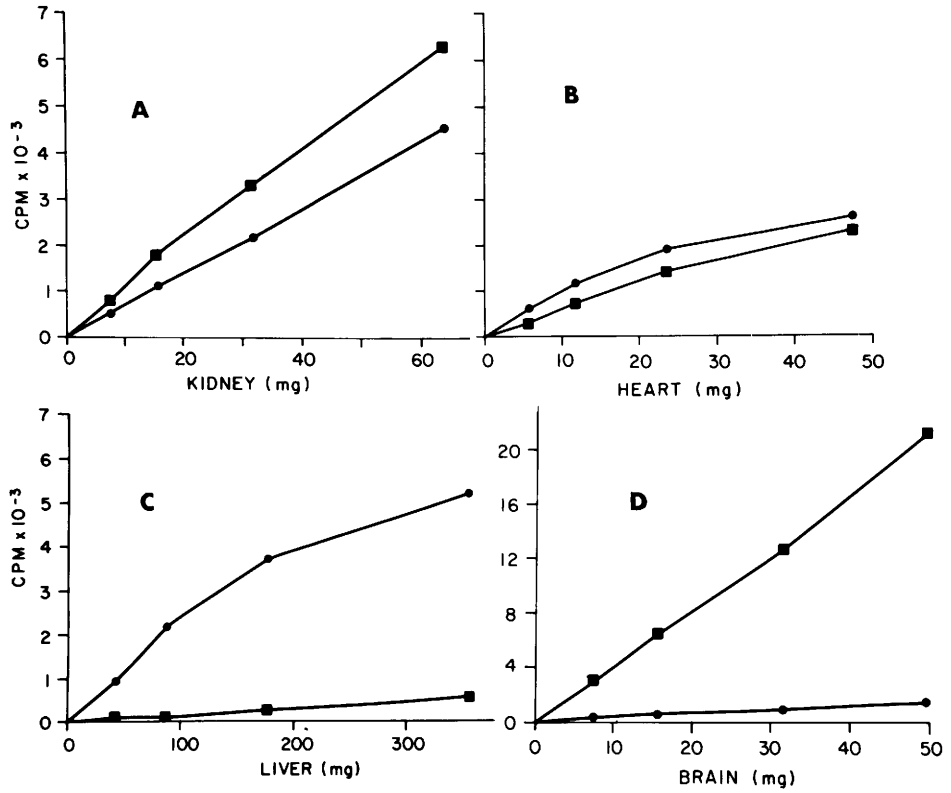

FIG. 1. Plot of the counts per minute (cpm) from $^{14}CO_2$ formed from D-lactate (●), or L-lactate (■), as a function of the amount of: (A) kidney (0–64 mg), (B) heart (0–48 mg), (C) liver (0–360 mg), (D) brain (0–50 mg) homogenates in reaction flasks incubated at 37°C for 20 min. Linear regression analysis of these data provide the following results; regression equations for cpm vs tissue amount, (r) and P values for each tissue and isomer: kidney 98(×) + 82 (0.991), $P < 0.001$ for L- and 71(×) − 15 (0.998), $P < 0.001$ for D-; heart 48(×) + 90 (0.991), $P < 0.001$ for L- and 79.7(×) + 117 (0.988), $P < 0.05$ for D-; liver 15(×) + 35 (0.991), $P < 0.001$ for L- and 21.1(×) + 78 (0.997), $P < 0.01$ for D-; brain 427(×) − 229 (0.999), $P < 0.001$ for L- and 29(×) + 164 (0.983), $P < 0.05$ for D-.

of these two isomers (D/L) was about 17 for homogenates and about 6 for slices. With both systems the D isomer was oxidized at a far greater rate than the L isomer. Thereby in order to avoid the problem of transport into the slices and because of ease of manipulation we utilized homogenates for the studies reported in this paper. Also, since brain slices are very fragile and function much like homogenates in the incubation system used, greater uniformity amongst tissues was achieved with homogenates.

Figure 3 represents the data for a specific amount of tissue from Fig. 2 with the specific activity (in cpm·g^{-1}·min^{-1}) for each tissue and isomer. A ratio over 1 indicates that L-lactate is being catabolized at a greater rate than the D isomer. The small amount of the L-lactate contamination of D-lactate (about 5% maximum from our chromatographic studies) causes a large effect on this ratio in brain tissue, due to the high activity for L-lactate oxidation and relatively low activity for D-lactate oxidation. If the contamination of the D-lactate by the L isomer is considered, it is apparent that the 14.4 ratio shown (legend for Fig. 3) for brain is actually too low. Corrections for the contamination change the ratio to approximately 50. Corrections for other tissues are not significant. The fact that brain showed this high specific activity for the L isomer has potentially significant implications involving

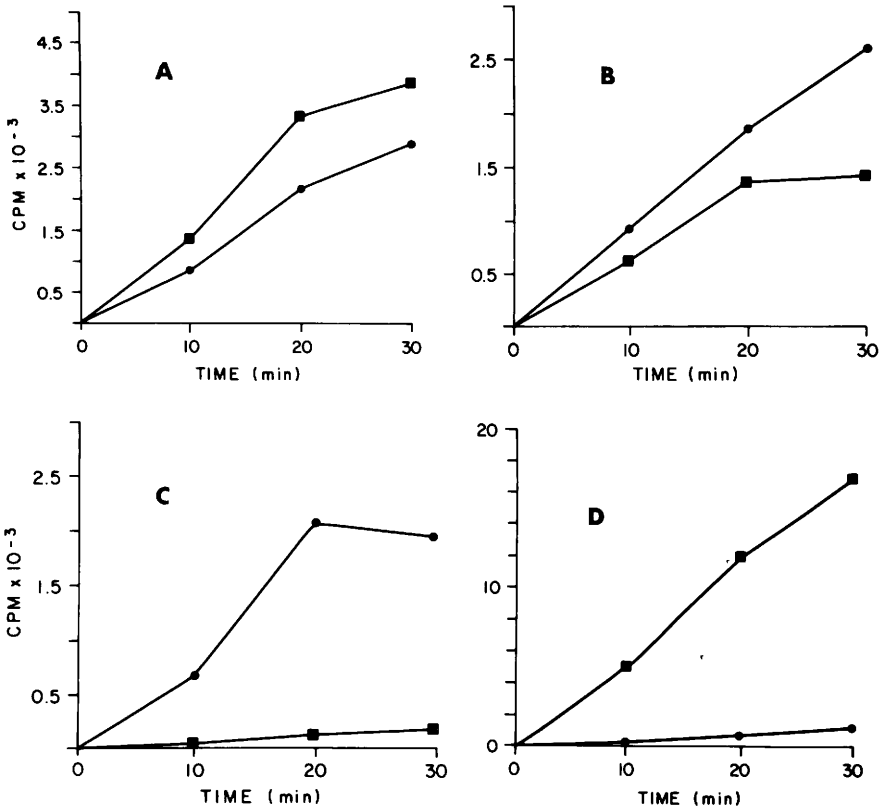

FIG. 2. Plot of cpm from $^{14}CO_2$ formed from D-lactate (●), or L-lactate (■), as a function of time in minutes for incubation of homogenates of: (A) kidney (32 mg), (B) heart (24 mg), (C) liver (90 mg), (D) brain (32 mg) in reaction flasks incubated at 37°C for 0–30 min. Linear regression analysis of these data provide the following results; regression equations for CPM vs time, (r) and P for each tissue and isomer: kidney 136(×) + 106 (0.980), $P < 0.05$ for L- and 100(×) − 15 (0.966), $P < 0.01$ for D-; heart 50(×) + 102 (0.960), $P < 0.05$ for L- and 87(×) + 44 (0.998), $P < 0.01$ for D-; liver 5.75(×) − 0.5 (0.995), $P < 0.01$ for L-; and 75(×) + 49 (0.966), $P < 0.05$ for D-; brain 579(×) − 121 (0.998), $P < 0.01$ for L- and 41.5(×) − 9 (0.999), $P < 0.001$ for D-.

neurological disturbances caused by abnormally elevated blood levels of D-lactate (17, 18). Accumulation of D-lactate in brain has been suggested to be due to a lack of adequate D-2-hydroxyacid dehydrogenase (17). Oxidation of D-lactate is catalyzed by a D-2-hydroxyacid oxidoreductase (EC 1.1.99.6) (23). Purification of this non-NAD-linked dehydrogenase showed that it was a mitochondrial bound enzyme of low activity (24). Since D-lactate does pass the blood–brain barrier (25) the elevated levels may interfere with normal catabolism of the L isomer. The overgrowth of certain bacteria in human intestine has the potential for increasing blood concentration of the D isomer (17, 18).

A similar correction for the D contamination of the L isotope used for measurement of L-lactate oxidation in liver homogenates, indicates that L-lactate catabolism to CO_2 was virtually nonexistent and the L/D ratio from the data in Fig. 3 is even smaller than shown. The other tissues shown in Fig. 3 are affected only marginally by isotope contamination corrections.

Under the conditions used in this study it was apparent that with exception of L-lactate metabolism in liver, all the tissues studied in depth (kidney, heart, liver, and brain) possessed active systems for both L-lactate and D-lactate metabolism in homogenates. Furthermore, metabolism of D-lactate, perhaps

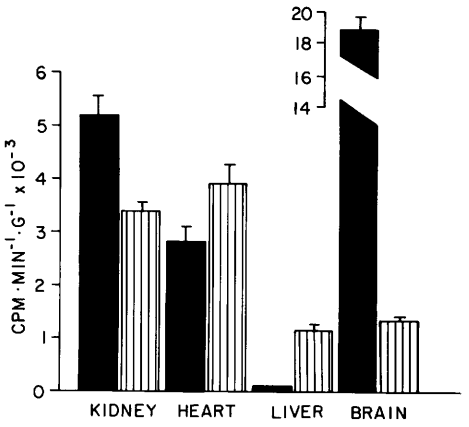

FIG. 3. Relative rates of oxidation to $^{14}CO_2$ for D-lactate ▥ and L-lactate ■ in cpm · g^{-1} of tissue (wet weight) · min^{-1} for the same weight of tissue homogenates as in Fig. 2 incubated at 37°C for 20 min. Bars represent $\bar{X}$ + SE for three experiments. (a) Kidney significantly different $^{14}CO_2$ formation between D- and L-lactate at $P < 0.01$. For the D isomer, significantly different against liver and brain at $P < 0.001$; nonsignificant against heart. For the L isomer significantly different against liver and brain at $P < 0.001$ and heart at $P < 0.005$. The ratio of L/D is 1.55 for kidney. (b) Heart not significantly different $^{14}CO_2$ formation between D- and L-lactate. For the D isomer, significantly different against liver and brain at $P < 0.001$. For the L isomer, significantly different against liver and brain at $P < 0.001$. The ratio of L/D is 0.73 for heart. (c) Liver significantly different $^{14}CO_2$ formation between D- and L-lactate at $P < 0.001$. For the D isomer not significantly different against brain. For the L isomer, significantly different against brain at $P < 0.001$. The ratio of L/D is 0.059 for liver. (d) Brain significantly different $^{14}CO_2$ formation between D- and L-lactate at $P < 0.001$. The ratio of L/D is 14.4 for brain.

surprisingly, was more active than that of L-lactate in liver while not statistically different in heart homogenates. This appears to be in contradiction to a number of previous studies in which D-lactate oxidation (9, 11) or utilization (10, 13) was low when compared to that of L-lactate. In one study, however, it was observed that liver oxidized D-lactate as well or better than L-lactate (14). Differing experimental and/or dietary conditions could account for the differences. In our experiments the animals were fasted 2 days prior to removal of tissues, to lower liver concentration of glycogen. This reduced the endogenous L-lactate concentration in liver. Thus, they were in a glucogenic state and possibly large amounts of L-lactate were being converted to glucose instead of being oxidized in the homogenates. Exton and Park (26) found that perfused livers from fasted rats synthesized more than twice as much carbohydrate from L-lactate than from D-lactate. Accordingly, the relatively high degree of D-lactate oxidation observed in our study may be related to the nutritional state of the animals.

A factor which should influence relative rates of radioactive D- and L-lactate oxidation in experiments reported here would be endogenous levels of nonradioactive D- and L-lactate in the homogenates and, consequently, carrier levels of nonradioactive D- and L-lactate added to the homogenates (Tables I and II). Although no measurable amount of endogenous D-lactate was found in the homogenates of brain, liver, kidney, and heart, a significant amount of endogenous L-lactate was present (Table I). Accordingly, in all experiments in which radioactive D- and L-lactate oxidation were compared, the incubation flasks for D-lactate oxidation were fortified with amounts of unlabeled D-lactate calculated to equal the amount of endogenous L-lactate that was present. It is evident from Table II data that this carrier D-lactate could significantly alter the rate of radioactive D-lactate oxidation and that the reported data comparing radioactive D- and L-lactate catabolism could be strongly influenced by the amount of this carrier D-lactate added. However, the L-lactate concentration did change during an incubation, particularly with liver homoge-

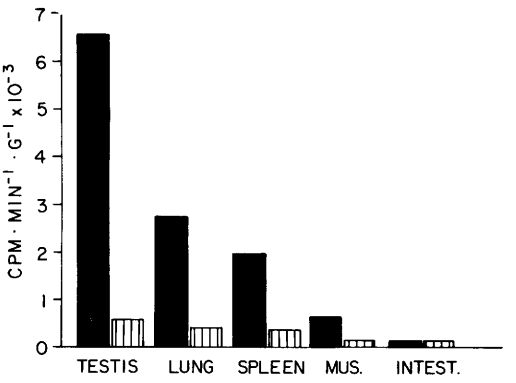

FIG. 4. Relative rates of oxidation to $^{14}CO_2$ for D-lactate ▥ and L-lactate ■ in cpm · g^{-1} of tissue (wet weight) · min^{-1} for tissue homogenates incubated at 37°C for 20 min. Bars represent $\bar{X}$ for two experiments.

nates, where the concentration increased significantly. In other tissues only very small changes in concentration occurred during incubation. Therefore, the amount of carrier D-lactate added to incubation flasks may not have always been optimal for achieving a concentration equal to that of endogenous L-lactate. Also, the presence of endogenous L-lactate in flasks in which D-lactate oxidation was measured and the absence of endogenous, and carrier D-lactate in flasks in which L-lactate oxidation was measured could have contributed to a more conservative measure of radioactive D-lactate oxidation, and a less conservative measure of radioactive L-lactate oxidation.

Based on the reasonable assumption that the oxidation of both lactate isomers leads to pyruvate, the observed differences for oxidation of the isomers would reside largely either in the relative activities of their respective dehydrogenases or in transport differences resulting from the subcellular localization of these enzymes (cytosolic for L-lactate and mitochondrial for D-lactate). Conceivably, cellular disruption may also dilute metabolites or effectors involved in lactate metabolism. If, in addition, a lactate racemase was active in any of the tissues investigated, it too could markedly influence the oxidation rates for D- and L-lactate. There is controversey regarding the existence of a mammalian lactic acid racemase (27–32).

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