Acta Medica et Biologica
Vol. 34, No. 3, 93-102, 1986

EXPIRATION OF RADIOACTIVE CARBON DIOXIDE BY RATS AFTER ADMINISTRATION OF ISOTOPIC LACTATE AND ACETATE

SHOUGO KUZE, M. D.,* YUSUKE ITO, M. D.**
AND
TATSURO MIYAHARA, Ph. D.***

**Associate professor, Department of Anesthesiology,*
Toyama Medical and Pharmaceutical University, Toyama, Japan
***Professor, Department of Anesthesiology,*
Toyama Medical and Pharmaceutical University, Toyama
****Associate professor, Faculty of Pharmaceutical Science*
Toyama Medical and Pharmaceutical University, Toyama

(Received May 22, 1986)

ABSTRACT

To clarify the oxidation of lactate and acetate contained in lactated and acetated Ringer's solutions, carbon dioxide productions from these compounds were investigated following a single intravenous injection of 1 mmol • kg^{-1} of [$1\text{-}^{14}C$] labelled DL-sodium lactate, L-sodium lactate or sodium acetate in unanesthetized rats. For DL-lactate, cumulative expired $^{14}CO_2$ rates of total radioactivities of administered ^{14}C were, raspectively, 16%, 37%, 63%, 70%, and 72%, at 15 minutes, 30 minutes, one hour, 2 hours and 6 hours following injection. These cumulative rates following DL-lactate administration were singnificantly lower for the first 40 minutes, compared with L-lactate and were only higher for the first 15 minutes, compared with acetate. These rates following DL-lactate administration were lower for doses of 1 mmol • kg^{-1} than for doses of 1 μmol • kg^{-1} and also lower in fasting rats than in non-fasting animals. These phenomena were not observed for acetate.

INTRODUCTION

Lactated or acetated Ringer's solutions are commonly used for hydration during anesthesia and surgery. However, metabolisms of the lactate and acetate contained in

these solution are not well understood. The purpose of this experiment, therefore, was to investigate the rates of expired $^{14}CO_2$ and the distributions of ^{14}C in the body following the intravenous injection of isotopic sodium lactate and sodium acetate into rats. With regard to stereoisomers, lactates were studied using DL-Lactate of racemic form and L-lactate, the natural form found in the human body.

METHOD

The experiments were carried out on unanesthetized male Wistar rats of about 220 grams body weight, which were usually fed until 9 o'clock on the morning of the start of each experiment. Fasting rats were deprived of food for 24 hours prior to the experiment, and given only water.

The following isotopic materials were purchased from New England Nuclear (Boston, Mass.): DL-[1-^{14}C]-sodium lactate (DL−$CH_3CH \cdot OH\ ^{14}COONa$, its specific activity was 45.4 mCi • $mmol^{-1}$), L-[1-^{14}C]-sodium lactate (L-$CH_3CH \cdot OH^{14}COONa$, 8.6 mCi • $mmol^{-1}$) and [1-^{14}C]-sodium acetate ($CH_3{}^{14}COONa$, 56.0 mCi • $mmol^{-1}$). Thes tracers were diluted with distilled water in case of injection doses of μmol • kg^{-1} of rat weight or were mixed with each non-radioactive carrier in the case of 1 mmol • kg^{-1} which had a radioactivity level of 50 μCi per 2 ml. Finally these solution were adjusted to pH 7.4 The animals were injected by a bolus injection through a tail vein with 0.2 ml of the above solutions per 100 grams rat weight.

Each rat was placed in an air tight container of 2 litres capacity, perfused with room air at a constant rate of 300 ml per minute by two pumps providing blowing and suction by which normal atmospheric pressure was maintained inside the container. Rats in containers were not restricted and were given water without food during the whole duration of CO_2 collection. The perfusing air was allowed to pass through 99 % ethanolamine that quantitatively absorbed the expired CO_2 and was changed at 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes and at 1.5, 2, 3, 4, 5 and 6 hours after the injection. With the liquid scintillator of Bray's solution[3], the radioactivity of each ethanolamine was determined by liquid scintillation counting (Aloka LSC-900).

At 6 hours the animals were anesthetized with an intraperitoneal injection of thiamylal. After blood samples were taken, the rats were sacrificed by decapitation. The urine excreted over the course of 6 hours including that in the urinary bladder was analysed for ^{14}C-radioactivity. In addition, various organs and tissues taken from the rats were subjected to combustion by sample oxidizer (Aloka ASC-112). ^{14}C-Radioactivities (disintegrations per minute : dpm) per gram of tissue and the distrbution of ^{14}C were determined.

RESULTS

1. Determination of ^{14}C from ^{14}C-lactate and -acetate after the injection of 1 mmol • kg^{-1}.

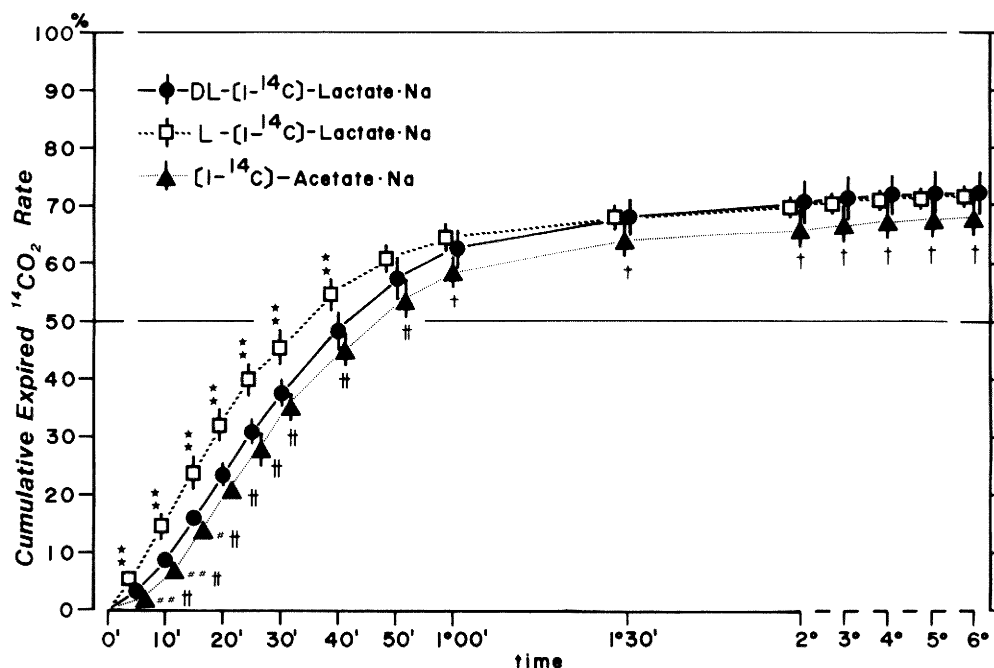

Fig. 1. Mean cumulative expired $^{14}CO_2$ rates ($\pm$SD) to total amount of administered ^{14}C following 1 mmol $\cdot$ kg^{-1} of [1-^{14}C]-labelled DL-lactate, L-lactate and acetate. n=7 in each group. The significant differences between DL-lactate and L-lactate are indicated as ** ($p<0.01$), those between DL-lactate and acetate as # ($p<0.05$) and ## ($p<0.01$), and those between L-lactate and acetate as+ ($p<0.05$) and ++ ($p<0.01$).

a. The cumulative expired $^{14}CO_2$ rates to total activities of administered ^{14}C (Fig. 1).

For DL-lactate, 16% at 15 minutes, 37% at 30 minutes, 63% at one hour, 70% at 2 hours and 72% at 6 hours were expired as $^{14}CO_2$. In DL -lactate, these rates were significantly lower for the first 40 minutes, compared with L-lactate. Thereafter, until the end of the experiment at 6 hours, there were no singnificant differences between DL-lactate and L-lactate. These rates for DL-lactate were only higer than those for acetate over the first 15 minutes, and afterwards, up to 6 hours, there were no significant differences between DL-lactate and acetate. During the whole six hours, however, these rates were always higher for L-lactate than for acetate.

b. Urinary excretion of ^{14}C over 6 hours.

Urinary excretion rates of administered ^{14}C were 1.3$\pm$0.7% (mean$\pm$standard deviation) for DL-lactate, 1.5$\pm$0.8% for L-lactate and 1.7$\pm$0.6% for acetate, showing no significant difference.

c. ^{14}C-Radioactivities per gram of tissues remaining after $^{14}CO_2$ collection for 6 hours (Fig. 2).

For DL-lactate, liver and pancreas showed the highest activites while brain and

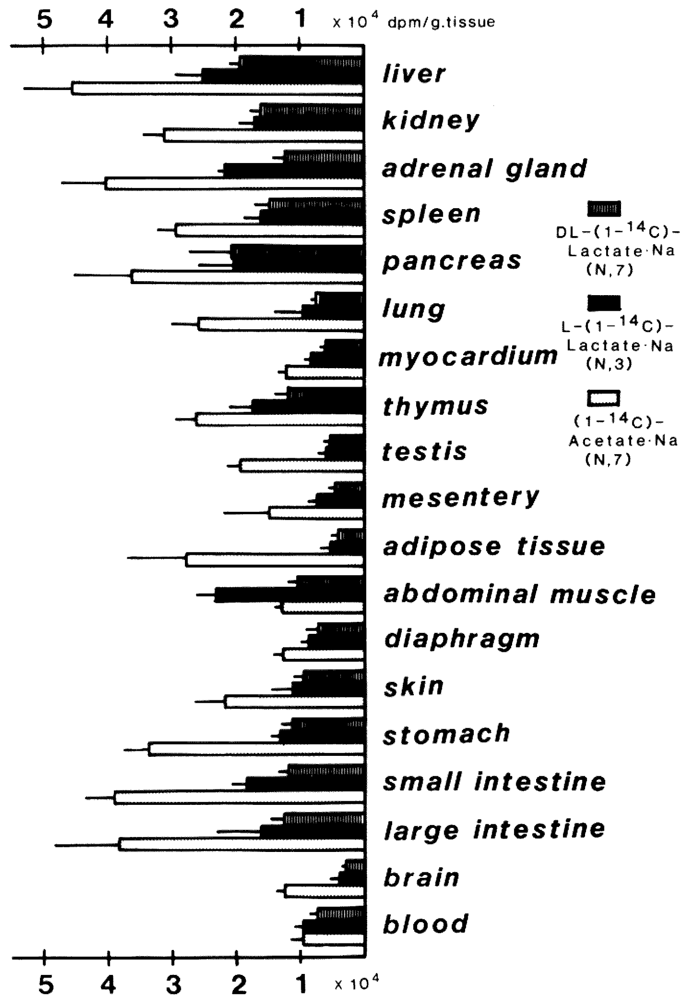

Fig. 2. Mean ^{14}C-radioactivities (+SD) remaining per gram of various tissues after $^{14}CO_2$ collection over 6 hours.

adipose tissue had the lowest. In L-lactate, as in DL-lactate, liver, abdominal muscle, adrenal gland and pancreas were in the highest group while brain and adipose tissue were in the lowest. For acetate every organ except blood had a higher activity, compared with lactate. Liver, adrenal gland, stomach and small intestine showed relatively high activities while the activities in the brain, myocardium, diaphragm and abdominal muscle were relatively low, and in blood they were the lowest.

2. Influence of dose level on expired $^{14}CO_2$ rates (Table 1).

With 1 μmol • kg^{-1} of DL−lactate, cumulative expired $^{14}CO_2$ rates were significantly higher over the course of the whole 6 hours, compared with a dose 1 mmol • kg^{-1}. That

Table 1. Influence of dose level on cumulative expired $^{14}CO_2$ rates[a]

time	DL-Lactate		L-Lactate		Acetate	
	1 μmol • kg^{-1}	1 mmol • kg^{-1}	1 μmol • kg^{-1}	1 mmol • kg^{-1}	1 μmol • kg^{-1}	1 mmol • kg^{-1}
15 min.	25 ± 6	16 ± 2**	27 ± 7	24 ± 3	18 ± 2	14 ± 1**
30 min.	49 ± 7	37 ± 2**	51 ± 8	46 ± 3	40 ± 4	35 ± 3*
1 hr.	73 ± 3	63 ± 3**	73 ± 4	65 ± 3**	63 ± 2	59 ± 3*
2 hrs.	81 ± 3	70 ± 4**	80 ± 4	70 ± 2**	69 ± 2	66 ± 2
6 hrs.	83 ± 3	72 ± 4**	82 ± 4	72 ± 2**	71 ± 2	69 ± 3

[a]per cent (mean±SD) of total radioactivities of administered ^{14}C, n=7 in each group.
The significant differences between 1 μmol•kg^{-1} and 1 mmol • kg^{-1} are indicated as * ($p<0.05$) and** ($p<0.01$).

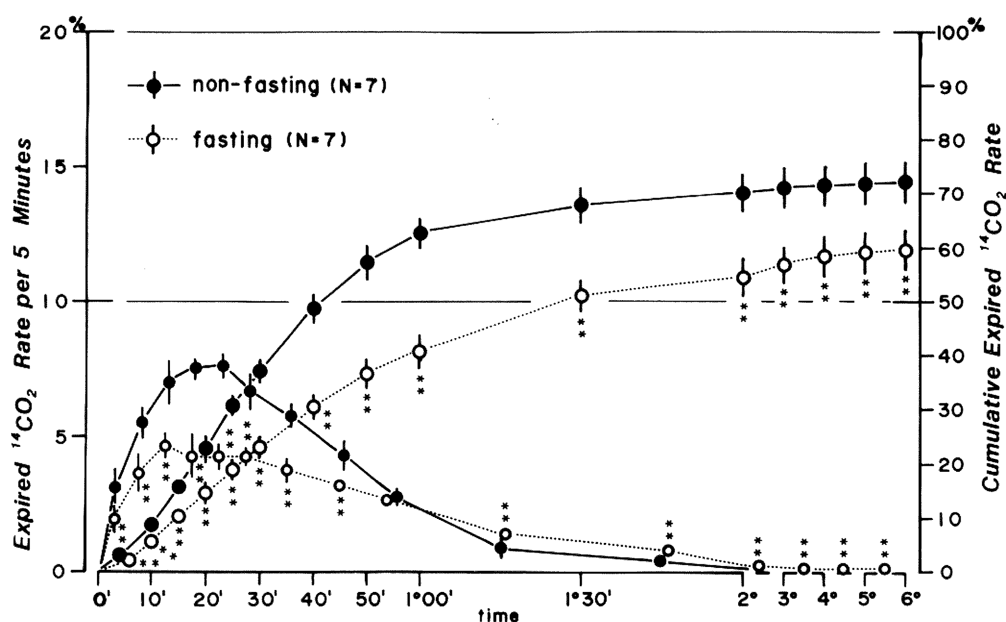

Fig. 3. Mean expired $^{14}CO_2$ rates (±SD) following administration of 1 mmol • kg^{-1} of DL-[1-^{14}C]-sodium lactate in non-fasting and fasting rats. The significant differences between the two groups of rats are indicated as **($p<0.01$).

is, with the smaller dose, the rates were 25% at 15 minutes, 49% at 30 minutes, 73% at one hour, 81% at 2 hours, finally reaching 83% at 6 hours. In L-lactate, differences were not observed over the first 30 minutes, but afterwards until the end of 6 hours these expired rates were higher for the 1 μmol • kg^{-1} dose, while in acetate these rates were only higher over the first one hour at a dose of 1 μmol • kg^{-1}, afterward becoming almost the same as the rates for 1 mmol • kg^{-1}.

3. Effects of fasting on expired $^{14}CO_2$ rates and ^{14}C-radioactivities remaining in tissues.

Production of $^{14}CO_2$ following the administration of 1 mmol • kg^{-1} DL-lactate were

strikingly depressed in fasting rats, compared with non-fasting rats (Fig. 3). In fasting rats the rates were 10% at 15 minutes, 23% at 30 minutes, 41% at one hour, 55% at 2 hours and reached only 60% at 6 hours. Fig. 4 illustrates the ^{14}C-radioativities ramaining in various organs and tissues at 6 hours after DL -lactate administration in non-fasting and fasting rats. These activities were always 3 to 4 times higher in fasting, compared with non-fasting animals. Similar phenomena were observed after L-lactate administr-tion. Therefore, in both DL- and L-lactate-treated rats the fasting state inhibited $^{14}CO_2$ production from lactates and preserved ^{14}C in the body.

$^{14}CO_2$ production after acetate administration in non-fasting and fasting rats is shown

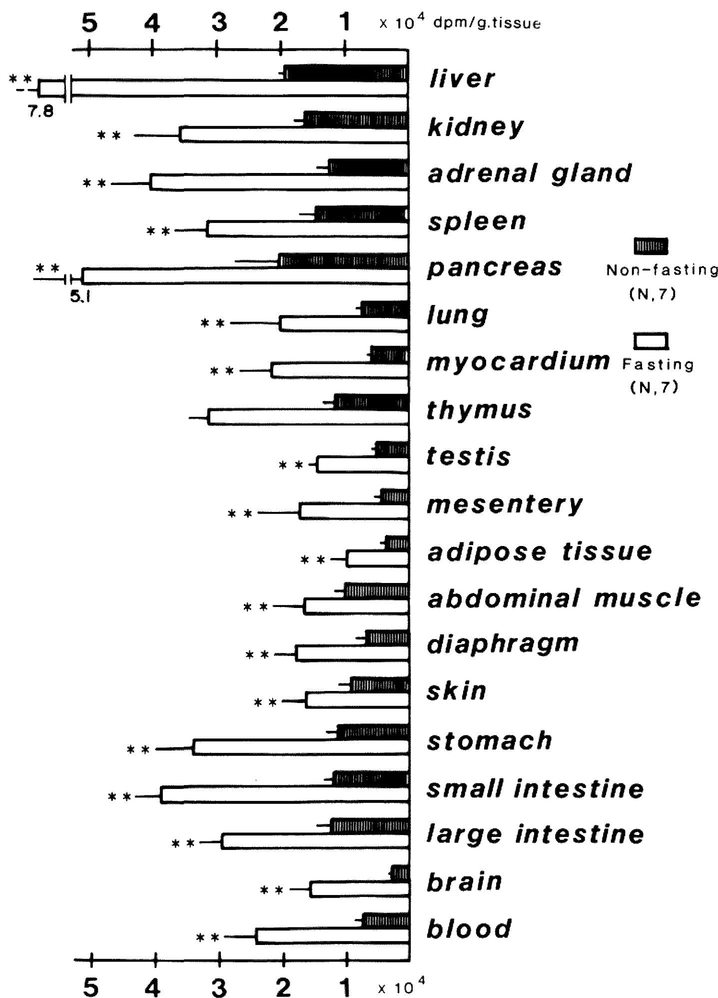

Fig. 4. Mean ^{14}C-radioactivities (+SD) per gram of tissues at 6 hours following administration of 1 mmol • kg^{-1} of DL-[1-^{14}C]-sodium lactate to non-fasting and fasting rats. The significant differences between the two groups of rats are indicated as** ($p<0.01$).

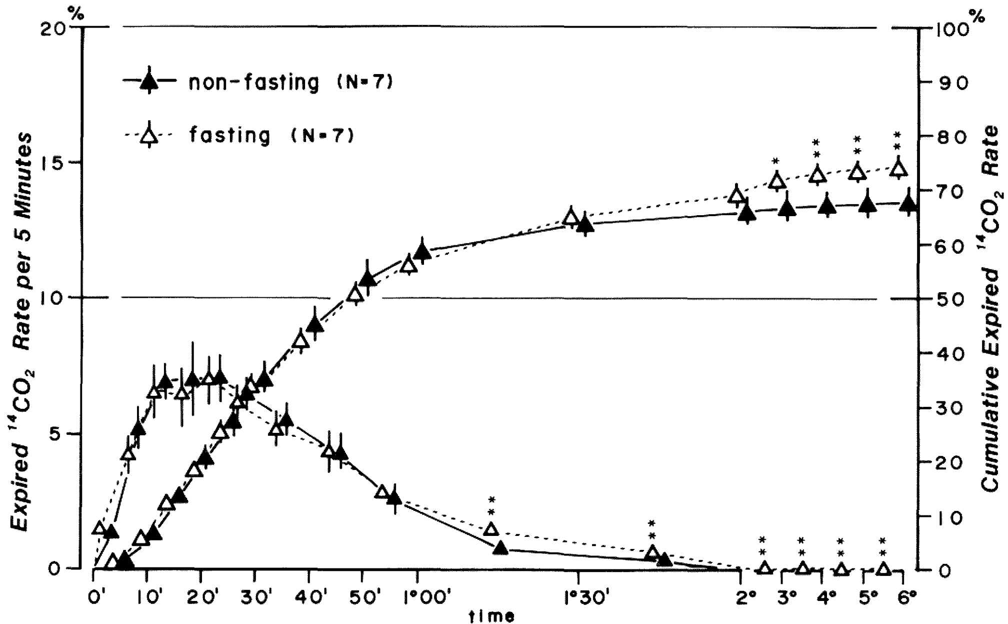

Fig. 5. Mean expired $^{14}CO_2$ rates (±SD) following administration of 1 mmol • kg^{-1} of [1-^{14}C]-sodium acetate to non-fasting and fasting rats. The significant difference between the two groups of rats are indicated as* ($p<0.05$) and ** ($p<0.01$).

in Fig. 5. With acetate, however, fasting had no effect upon expired $^{14}CO_2$ rates for the first 2 hours. After 2 hours and up until 6 hours these rates were slightly higher in fasting than in non-fasting animals. Fasting also had hardly any effect upon ^{14}C-radioactivity remaining in organs and tissues after ^{14}C-acetate administration.

DISCUSSION

Ringer's solution containing lactate has been widely used for hydrating patients during operations for the following reasons. Lactated Ringer's solution has a similar electrolyte composition to the extracellular fluids and produces an equi-molar bicarbonate ion from the lactate metabolism (Hartmann et al.[11] Hartmann[12]). Lactate, however, has two stereoisomers, L- and D-form, which have different metabolic rates (Cori and Cori[7]) . Since D-lactate cannot be metabolized by any enzymes in the central nervous system (Brin[4]), D-lactic acidosis which causes severe neurological and psychological disturbances, has attracted serious attention recently (Oh et al.[16]). In Japan, lactated Ringer's solutions available on the market contain DL-lactate, its racemic form. A company, however, has started to offer the solution with just L-lactate. In contrast with this development, replacing lactate with acetate in the solution has also been considered. Mudge, Manning and Gilman[15], for example, have proposed using acetate. This idea is supported by the fact that acetate metabolism in human is recently becoming well-

understood and is also commonly used in kidney dialysis solution.

The concentration of the organic acid salts in both lactated and acetated Ringer's solution is 28 mmol • L^{-1}. For this study, we have examined the effects of two different dose levels of the organic acid salts in question, i. e., 1 μmol • kg^{-1} and 1 mmol • kg^{-1} of body weight, on the oxidation of these acids. We have chosen these two levels because under any ordinary clinical conditions, not more than 1 mmol of such acids per kilogram of body weight would be administered at once.

Most of L-lactate either administered from outside or produced in the body is metabolized in the liver and the rest in kidney, muscle, heart, and brain (Ritz et al.[17)]). By the action of NAD^+ and L-lactate dehydrogenase (L-LDH), L-lactate is converted to pyruvate which, in turn, is oxidized through the TCA cycle and finally expired as CO_2. L-lactate is also mobilized as a substrate for gluconeogenesis and completed Cori's cycle, making a circle from liver glycogen to blood glucose to muscle glycogen to blood lactate and back to liver glycogen (Cori and Cori[7)]). It was reported that the maximum metabolic rate of lactate in human liver was 3400 mmol per day (Alberti[1)]).

There is still no clear understanding of D-lactate metabolism in humans. It was once believed that D-lactate was converted to L-form by lactate racemase or catalysed by D-LDH. These enzymes, however, have not been found in mammalian systems (Coran[6)]). Presently, a non-specific enzyme, D-2-hydroxy acide dehydrogenase, is considered to be responsible for producing pyruvate from D lactate (Cammack,[5)] Giesecke et al.[9)]). It has also been believed that D-lactate is slowly metabolized at one quarter of the metabolic rate for L-lactate and that 30% of the ingested amount is excreted in urine (Cori and Cori[7)]). Indeed, Table 1 shows that cumulative expired $^{14}CO_2$ rates in DL-lactate were significantly lower in the group treated with 1 mmol • kg^{-1} dose than the group treated with 1 μmol • kg^{-1} whereas in L-lactate there was no difference for at least the first 30 minutes between two groups given the same dose levels. On the other hand, for the first 40 minutes, expired $^{14}CO_2$ rates with 1 mmol • kg^{-1} were lower in the DL-lactate group than in the L-lactate group as shown in Fig. 1. These findings indicated that the DL-lactate was metabolized slower than the L-form alone.

Although the DL-form was metabolized slowly, it had the same expiration rate as the L-form at 1 μmol • kg^{-1} dose level during the complete CO_2 collection of 6 hours (Table 1) and also the same rate at 1 mmol • kg^{-1} dose level after 50 minutes of injection (Fig. 1.). In addition, it was found that the amount of radioactivity excreted in urine was less than 2% of the total radioactivity administered to both the DL- and L-lactate group. Giesecke and Fabritius[9)] observed $^{14}CO_2$ expiration after 6 hours of intraperitoneal injection of ^{14}C-D-lactate. They found that the radioativities in $^{14}CO_2$ were 84% of the total radioactivity in the group of rats which was fed with lactate-free diet and 82% from another group fed with 5% DL-lactate diet. Amounts of ^{14}C found in urine were 3% of the total in both groups. Thus, their results were very close to ours discussed above. Therefore, it can be concluded that utilization of D-lactate is dependent on dose and at

the dose levels used in this experiment it is not as low as we have believed before.

It should be noted that the rate of $^{14}CO_2$ expiration does not necessarily concur with the metabolic rate of labelled compunds. The group administered with ^{14}C-acetate showed a lower $^{14}CO_2$ expiration rate than the group given L-^{14}C-lactate as seen in Fig. 1, but by no means is acetate metabolism slower than L-lactate metabolism.

Lactate is metabolized mainly in the liver, whereas actate is utilized mainly in the peripheral tissues (Giesecke et al.[9] Harper et al.[10]). Acetate is converted to acetyl-CoA by acetate thiokinase in the presence of CoA and ATP (Ballard[2]). This enzyme activity is especially high in the liver, myocardium, kidney cortex and brain, but low in skeletal muscle. Despite the low activity, skeletal muscle has the highest capacity of utilizing acetate due to its total activity of the enzyme (Ballard[2]). It has been reported that the metabolic rate of acetate in human is 300 mmol • hour^{-1} which is twice as much as L-lactate (Lundquist[14]). After entering the TCA cycle, acetyl-CoA is oxidized to CO_2 and H_2O. It also participates in both lipid and amino acid sythesis (Lundquist[14]). However, it is uncertain whether acetate may be involved in gluconeogenesis (Deuer and Milhorat[8]).

There was a striking contrast in the influence of fasting on oxidations between lactate and acetate. In the case of lactate, the fasting mobilized lactate for gluconeogenesis through Cori's cycle (Cori et al.[7]) and clearly inhibited the expiration of $^{14}CO_2$ (Fig. 3). As a result, the amount of ^{14}C in various organs from the fasting group was more than two times higher compared to the non-fasting group (Fig. 4.) On the other hand, both fasting and non-fasting groups administered with acetate had no difference in the cumulative expired $^{14}CO_2$ rate up to 2 hours after injection (Fig. 5) and the effects of fasting were not observed at al. After 2 hours, however, the fasting group seemed to have a slightly higher expiration of $^{14}CO_2$ than non-fasting group. We assumed that some acetate had been incorporated into substances in metabolic pathway other than TCA cycle in both fasting and non-fasting groups. And because of the decreasing energy supply, these metabolites, if only a small amount, might be taken into the TCA cycle, especially in fasting animals.

The cumulative expired $^{14}CO_2$ rates after the administration of labelled lactate and acetate did not show the rate of metabolism of labelled materials. However, the data we obtained yielded information on the rate at which oxidation participates in the metabolic processes of these substrates, as well as the rate at which isotopic carbon are eliminated following their administrations.

REFERENCES

1) Alberti, K. G. M. M. and Nattrass, M.: Lactic acidosis. *Lancet*, ii, 25-29, 1977.

2) Ballard, F. J.: Supply and utilization of acetate in mammals. *Am. J. Clin. Nutr.*, 25: 773-779, 1972.

3) Bray, G. A.: A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.*, 1: 279-285, 1960.

4) Brin, M.: The synthesis and metabolism of lactic acid isomers. *Ann. NY. Acad. Sci.,* **119**: 942-956, 1965.
5) Cammack, R.: Assay, purification and propertied of mammalian D-2-hydroxy acid dehydrogenase. *Biochem. J.,* **115**: 55-64, 1969.
6) Coran, A. G.: The effect of lactated Ringer's solution on blood and urine levels of lactated isomers. *J. Surg. Res.,* **11**: 450-453, 1971.
7) Cori, C. F. and Cori, G. T.: Glycogen formation in the liver from d- and l-lactic acid. *J. Biol. Chem.,* **81**: 389-403, 1929.
8) Deuer, H. J. Jr. and Milhorat, A. T.: On the alleged conversion of fat to carbohydrate. I. The metabolism of acetic acid. *J. Biol. Chem.,* **78**: 299-309, 1928.
9) Giesecke, D. and Fabritius, A.: Oxidation and excretion of D-lactic acid by rats. *Experientia,* **30**: 1124-1125, 1974.
10) Harper, P. V. Jr., Neal, W. B. and Hlavacek, G. R.: Acetate utilization in the dog. *Metabolism,* **2**: 62-68, 1953.
11) Hartmann, A. F. and Senn, M. J. E.: Studies in the metabolism of sodium r-lactate. I. Response of normal human subjects to the intravenous injection of sodium r-lactate. *J. Clin. Invest.,* **11**: 327-335, 1932.
12) Hartman, A. F.: Theory and practice of parenteral fluid administration. *J. A. M. A.,* **103**: 1349-1354, 1934.
13) Kusaka, M., and Ui, M.: Tracer kinetics analysis of Cori cycle activity in the rat: Effect of feeding. *Am. J. Physiol.,* **232**: E136-144, 1977.
14) Lundquist, F.: Production and utilization of free acetate in man. *Nature,* **193**: 579-580, 1962.
15) Mudge, G. H., Manning, J. A. and Gilman, A.: Sodium acetate as a source of fixed base. *Proc. Soc. Exptl. Bio. Med.,* **71**: 136-138, 1949.
16) Oh, M. S., Phelps, K. R., Traube, M. et al.: D-Lactic acidosis in a man with the short-bowel syndrome. *New Eng. J. Med.,* **301**: 249-252, 1979.
17) Ritz, E., and Heidland, A.: Lactic acidosis. *Clin. Nephrol.,* **7**: 231-240, 1977.