

Metabolism of ^3H - and ^{14}C -labelled lactate in starved rats

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1. [$2\text{-}^3\text{H},\text{U-}^{14}\text{C}$]- or [$3\text{-}^3\text{H},\text{U-}^{14}\text{C}$]-Lactate was administered by infusion or bolus injection to overnight-starved rats. Tracer lactate was injected or infused through indwelling cannulas into the aorta and blood was sampled from the vena cava (A–VC mode), or it was administered into the vena cava and sampled from the aorta (V–A mode). Sampling was continued after infusion was terminated to obtain the wash-out curves for the tracer. The activities of lactate, glucose, amino acids and water were followed. 2. The kinetics of labelled lactate in the two modes differed markedly, but the kinetics of labelled glucose were much the same irrespective of mode. 3. The kinetics of ^3H -labelled lactate differed markedly from those for [$\text{U-}^{14}\text{C}$]lactate. Isotopic steady state was attained in less than 1 h of infusion of [^3H]lactate but required over 6 h for [$\text{U-}^{14}\text{C}$]lactate. 4. ^3H from [$2\text{-}^3\text{H}$]lactate labels glucose more extensive than does that from [$3\text{-}^3\text{H}$]lactate. [$3\text{-}^3\text{H}$]Lactate also labels plasma amino acids. The distribution of ^3H in glucose was determined. 5. Maximal radioactivity in ^3HOH in plasma is attained in less than 1 min after injection. Near-maximal radioactivity in [^{14}C]glucose and [^3H]glucose is attained within 2–3 min after injection. 6. The apparent replacement rates for lactate were calculated from the areas under the specific-radioactivity curves or plateau specific radioactivities after primed infusion. Results calculated from bolus injection and infusion agreed closely. The apparent replacement rate for [^3H]lactate from the A–VC mode averaged about 16 mg/min per kg body wt. and that in the V–A mode about 8.5 mg/min per kg body wt. The apparent rates for [^{14}C]lactate ('rate of irreversible disposal') were 8 mg/min per kg body wt. for the A–VC mode and 5.5 mg/min per kg body wt. for the V–A mode. Apparent recycling of lactate carbon was 55–60% according to the A–VC mode and 35% according to the V–A mode. 7. The specific radioactivities of [$\text{U-}^{14}\text{C}$]glucose at isotopic steady state were 55% and 45% that of [$\text{U-}^{14}\text{C}$]lactate in the A–VC and V–A modes respectively. We calculated, correcting for the dilution of ^{14}C in gluconeogenesis via oxaloacetate, that over 70% of newly synthesized glucose was derived from circulating lactate. 8. Recycling of ^3H between lactate and glucose was evaluated. It has no significant effect on the calculation of the replacement rate, but affects considerably the areas under the wash-out curves for both [$2\text{-}^3\text{H}$]- and [$3\text{-}^3\text{H}$]-lactate, and calculation of mean transit time and total lactate mass in the body. Corrected for recycling, in the A–VC mode the mean transit time is about 3 min, the lactate mass about 50 mg/kg body wt. and the lactate space about 65% of body space. The V–A mode yields a mass and lactate space about half those with the A–VC mode. 9. The area under the wash-out curve for [^{14}C]lactate is some 20–30 times that for [^3H]lactate, and apparent carbon mass is 400–500 mg/kg body wt. and presumably includes the carbon of glucose, pyruvate and amino acids, which are exchanging rapidly with that of lactate.

In the preceding paper (Katz *et al.*, 1981) we showed the marked dependence of the tracer kinetics of lactate on the sites of administration of the

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labelled lactate and sampling. We have also shown the large difference in the kinetics of $3\text{-}^3\text{H}$ - and $\text{U-}^{14}\text{C}$ -labelled lactate. In the present paper we present studies with lactate labelled in position 2 or 3

with ^3H and uniformly with ^{14}C , administered by continuous infusion or bolus injection, and the calculations of apparent replacement rates, mass of body lactate, recycling of lactate carbon and tritium, and the contribution of lactate to glucose synthesis. Results obtained by different modes of tracer administration and sampling are compared. The merits and limitations of ^3H and ^{14}C as tracers for lactate turnover *in vivo* are discussed.

Methods

Experimental design

Male rats of the Sprague-Dawley strain (body wt. 250–300 g) starved for 20 h were used. Two indwelling catheters were implanted in each rat 4–5 days before experiments, as previously described (Katz *et al.*, 1974b). In most experiments there were implanted a venous cannula terminating in the vena cava and an arterial cannula terminating in the aortic arch. In one series of experiments two arterial cannulae were implanted, one in the aortic arch and another through a femoral artery terminating in the descending aorta, just above the iliac bifurcation. The position of the catheter tips was ascertained at the end of the experiments. Three modes of tracer administration by primed continuous infusion were employed: (a) infusion into vena cava, sampling from the aortic arch (V–A mode); (b) infusion into aortic arch, sampling from the vena cava (A–VC mode); (c) infusion into lower aorta and sampling from the aortic arch (A–A mode). In bolus injection, injection and sampling was in either the V–A or the A–VC mode. In some experiments sampling and collection of blood was from the same cannula in the aortic arch. Rapid tracer injection in a volume of about 0.1 ml was followed by about 1 ml of saline to rinse the cannula. Collection of blood was begun within 3–4 s after injection. Results obtained by this procedure were identical with those obtained by the A–VC mode and are grouped together with those of the A–VC mode.

Labelled compounds

[2- ^3H]Lactate was prepared by reduction of pyruvate with NAD^3H essentially as described by Rognstad (1970). [3- ^3H]Lactate was prepared as described in the preceding paper (Katz *et al.*, 1981). [4- ^3H]Glucose was prepared as follows. DL-[2- ^3H]Lactate was made by reduction of pyruvate with NaB^3H_4 . NAD^3H was generated from [2- ^3H]lactate with lactate dehydrogenase, alanine aminotransferase and excess of glutamate. 1,3-Bisphosphoglyceric acid was reduced with NAD^3H to [1- ^3H]glyceraldehyde 3-phosphate by the appropriate enzymes. The labelled glyceraldehyde 3-phosphate was incorporated into [4- ^3H]fructose 6-phosphate by the action of transaldolase in the presence of

excess of fructose, which serves as dihydroxyacetone donor. The labelled fructose 6-phosphate was converted into [4- ^3H]glucose 6-phosphate by phosphohexose isomerase. The whole reaction sequence up to the hexose phosphate mixture was performed as a single step in one reaction mixture, with the use of commercially available enzymes, without isolating NAD^3H or any other intermediates. The mixture containing the hexose phosphates was hydrolysed with acid phosphatase, and glucose was isolated by paper chromatography. Other labelled compounds were purchased from New England Nuclear (Boston, MA, U.S.A.).

Degradation

Glucose formed from [2- ^3H]- and [3- ^3H]-lactate was isolated and degraded to obtain the ^3H content of positions 1, 6 and 4. ^3H on position 6 was determined as the formaldehyde dimedone derivative after periodate degradation as described by Bloom (1962). ^3H on position 1 was obtained as water after oxidation of glucose with glucose oxidase and catalase. The labelled water was separated by passage through tandem ion-exchange columns as described previously (Katz *et al.*, 1981). ^3H on C-4 was obtained by difference.

Results

We describe first the general characteristics of the kinetics of ^3H -labelled and ^{14}C -labelled lactate and of their products. Subsequently we present our calculations of the apparent replacement rates and mass, recycling and other parameters of lactate metabolism in the A–VC and V–A modes. In the two modes the patterns obtained with ^3H -labelled and ^{14}C -labelled lactate differ very markedly. The labelled products found in plasma derived from [^{14}C]lactate were, apart from $^{14}\text{CO}_2$ and pyruvate, which were not determined, glucose and amino acids. The amount of ^{14}C in oxo acids or other products was very low. The amino acid fraction was examined in a few trials by paper chromatography. Over half of the ^{14}C was present in alanine, and the rest nearly all in glutamine and glutamic acid. By far the greatest amount of activity from ^3H -labelled lactate was recovered in water, but there was significant incorporation of ^3H from both [2- ^3H]lactate and [3- ^3H]lactate into glucose. ^3H from [3- ^3H]lactate was incorporated into amino acids, but little if any from [2- ^3H]lactate.

Confusion infusion

In the preceding paper (Katz *et al.*, 1981, Fig. 2) we showed that approach to isotopic steady state in continuous infusion is quite rapid in both modes with [3- ^3H]lactate. This is also true for [2- ^3H]lactate.

Isotopic steady state is attained without priming within less than 30 min in the V-A mode and somewhat more slowly in the A-VC mode. On the other hand, with $[\text{U-}^{14}\text{C}]$ lactate the approach to constant specific radioactivity is very slow. An unprimed infusion would require some 6 h to attain a value within $\pm 10\%$ of the asymptote. The terminal slope is shallow, so that an increase in specific radioactivity for a 20–30 min interval is much the same as the experimental error (noise), so that it is difficult to ascertain whether a constant specific radioactivity has been obtained. With $[\text{U-}^{14}\text{C}]$ lactate we use a priming dose equivalent of 150–180 min, followed by 150 min of infusion. Since the glucose concentration in plasma is much greater and more stable than that of lactate, the specific radioactivity

of glucose can be measured with greater precision, and constant specific radioactivity of glucose is the best criterion of isotopic steady state.

In Fig. 1 we present the average specific radioactivities in lactate and glucose after primed infusion of $[\text{2-}^3\text{H}, \text{U-}^{14}\text{C}]$ - and $[\text{3-}^3\text{H}, \text{U-}^{14}\text{C}]$ -lactate in the conventional V-A mode (infusion into vena cava, sampling from the aorta), and in Fig. 2 in the reverse A-VC mode (infusion into aorta, sampling from the vena cava). After termination of the infusion, sampling was continued to obtain the wash-out curves. There are marked quantitative differences in the plateaus and slopes between the two modes (explored in more detail in the Discussion section), but the overall kinetic patterns and distinctions between the ^3H and ^{14}C specific-radioactivity curves

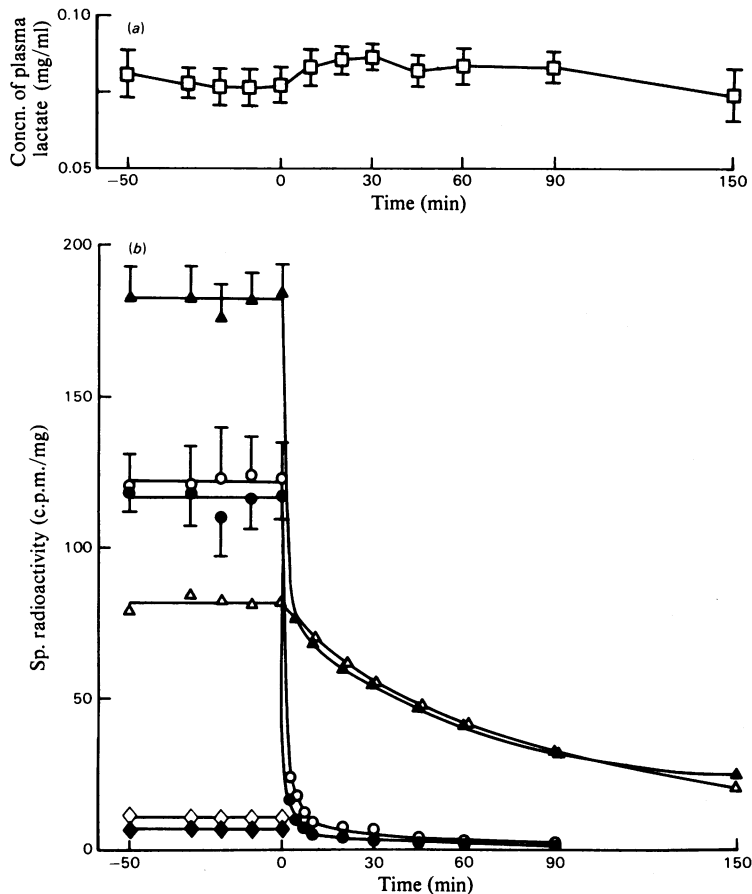


Fig. 1. Constant infusion of ^3H -labelled and $\text{U-}^{14}\text{C}$ -labelled lactate in the V-A mode

Average results of five experiments each with $[\text{2-}^3\text{H}]$ lactate and $[\text{3-}^3\text{H}]$ lactate and ten experiments with $[\text{U-}^{14}\text{C}]$ lactate are shown. Tracer was infused into the vena cava and sampling was from the aorta. Results are normalized to an infusion of 1000 c.p.m./min per kg body wt. Plasma specific radioactivity is expressed as c.p.m./mg of lactate or of glucose. The s.e.m. is shown when it exceeds $\pm 5\%$. (a) □, Concn. of plasma lactate. (b) Specific radioactivity of: ▲, $[\text{U-}^{14}\text{C}]$ lactate; ○, $[\text{2-}^3\text{H}]$ lactate; ●, $[\text{3-}^3\text{H}]$ lactate; △, ^{14}C -labelled glucose; ◇, glucose from $[\text{2-}^3\text{H}]$ acetate; ◆, glucose from $[\text{3-}^3\text{H}]$ acetate.

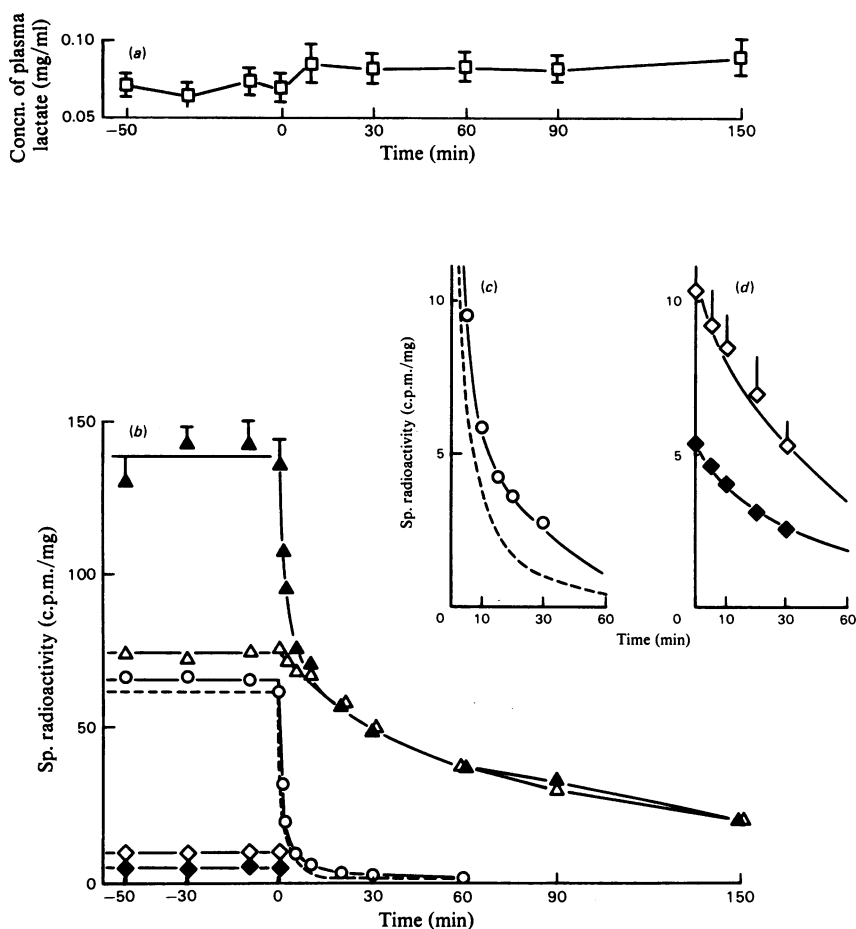


Fig. 2. Constant infusion of $[2\text{-}^3\text{H}]\text{lactate}$, $[3\text{-}^3\text{H}]\text{lactate}$ and $[\text{U}\text{-}^{14}\text{C}]\text{lactate}$ in the A-VC mode

Average results of four experiments with $[2\text{-}^3\text{H}]\text{lactate}$, five experiments with $[3\text{-}^3\text{H}]\text{lactate}$ and nine experiments with $[\text{U}\text{-}^{14}\text{C}]\text{lactate}$ are shown. Tracer was infused into the aorta and sampling was from the vena cava. Normalization and results are expressed as in Fig. 1. (a) \square , Concn. of plasma lactate. (b)–(d) Specific radioactivity of: \blacktriangle , $[\text{U}\text{-}^{14}\text{C}]\text{lactate}$; \triangle , ^{14}C -labelled glucose; \circ , $[2\text{-}^3\text{H}]\text{lactate}$ and $[3\text{-}^3\text{H}]\text{lactate}$; \diamond , glucose from $[2\text{-}^3\text{H}]\text{lactate}$; \blacklozenge , glucose from $[3\text{-}^3\text{H}]\text{lactate}$. (c) Enlarged scale for wash-out curves from $[^3\text{H}]\text{lactate}$; — — —, specific radioactivity of $[^3\text{H}]\text{lactate}$ corrected for recycling of ^3H . (d) Wash-out curves for ^3H -labelled glucose.

have similar features. There was a small difference in the curves for $[2\text{-}^3\text{H}]\text{lactate}$ and $[3\text{-}^3\text{H}]\text{lactate}$ in the V-A mode, whereas in the A-VC mode there was no statistically significant difference, and we have combined in all experiments the results for the two tracers. The incorporation into glucose was always substantially greater from $[2\text{-}^3\text{H}]\text{lactate}$ than from $[3\text{-}^3\text{H}]\text{lactate}$. This was also observed with rat hepatocytes *in vitro* by Rognstad & Wals (1976).

Of special interest are the wash-out curves. Those for $[2\text{-}^3\text{H}]\text{lactate}$ and $[3\text{-}^3\text{H}]\text{lactate}$ decayed rapidly, and within 1 h the specific radioactivities decreased to 1–2% of the plateau values. On the other hand with $[\text{U}\text{-}^{14}\text{C}]\text{lactate}$, after an initial rapid fall, the curves flattened out. At 3 h after the termination of infusion

the specific radioactivity of $[\text{U}\text{-}^{14}\text{C}]\text{lactate}$ in the V-A mode was 10–15% of the plateau values and was still higher in the A-VC mode. Exponential extrapolation of the tails indicated that 6–8 h would be required for the specific radioactivity to decline to 1% of that of the plateau. The areas under the ^{14}C wash-out curves were 20–30 times those for the ^3H wash-out curves. About 5 min after the termination of infusion the specific radioactivities of $[\text{U}\text{-}^{14}\text{C}]\text{lactate}$ and $[\text{U}\text{-}^{14}\text{C}]\text{glucose}$ became equal, and in both modes the specific radioactivities of lactate and glucose remained thereafter virtually identical. This indicates that plasma lactate was virtually all derived from circulating glucose, without dilution from glycogen, amino acids or tissue protein.

In Figs. 2(c) and 2(d) we present on an enlarged scale the specific radioactivities of glucose from $[2\text{-}^3\text{H}]\text{lactate}$ and $[3\text{-}^3\text{H}]\text{lactate}$ in wash-out. The two curves differ markedly, showing that the distribution of ^3H in glucose from $[2\text{-}^3\text{H}]\text{lactate}$ differs from that in glucose from $[3\text{-}^3\text{H}]\text{lactate}$. In both cases after 5–10 min the specific radioactivity of glucose exceeded that of lactate.

Fig. 3 presents the total radioactivities in plasma for the experiments of Fig. 2. The incorporation into amino acids is also shown in Fig. 3. At isotopic steady state the radioactivity in plasma glucose exceeds that in lactate for all tracers. In wash-out the radioactivity in ^3H -labelled glucose becomes very much greater than that in lactate.

Single injection

In the preceding paper (Katz *et al.*, 1981) we compared the injection of a bolus of $[3\text{-}^3\text{H}]\text{lactate}$ in the V–A and A–VC modes. It was shown that very early and rapid sampling, within seconds after injection, is essential to obtain correct measures of the areas under the curves. Rapid sampling is much easier if blood is collected from a large artery. We have thus in some experiments adopted a modification of the A–VC mode, with blood injected and sampled from the aorta. We detected no significant difference between the two procedures and present their results together. In Fig. 4 we present our average results with single injection in

this mode, reporting total rather than specific radioactivities in plasma.

Maximal specific radioactivity in the A–VC mode was attained 9–15 s after injection (as compared with 3–5 s in the V–A mode), the maximal specific radioactivity is one-fifth of that obtained in the A–V mode, and the initial decay is much slower (compare with Fig. 1 of Katz *et al.*, 1981). The specific-radioactivity curves for $[2\text{-}^3\text{H}]\text{-}$ and $[3\text{-}^3\text{H}]\text{-lactate}$ were essentially the same. The specific radioactivities of ^3H -labelled and ^{14}C -labelled lactate diverged at about 1 min after injection. The maximal radioactivity in water was attained within 1 min after injection in the A–VC mode, just as in the V–A mode (Katz *et al.*, 1980). The curves for the specific radioactivity of glucose showed a rather flat maximum in the ranges 2–4 min for $[^3\text{H}]\text{glucose}$ and 4–8 min for $[^{14}\text{C}]\text{glucose}$. In some experiments maximal radioactivity in $[^{14}\text{C}]\text{glucose}$ was attained at less than 3 min. The maximal specific radioactivities in amino acids from $[3\text{-}^3\text{H}]\text{-}$ or $[^{14}\text{C}]\text{-lactate}$ were attained very rapidly, less than 1 min after injection.

The turnover of lactate is very rapid. By 5 min after injection 75–80% of the total ^3H radioactivity in plasma is in water, 10–15% in glucose and/or amino acids, and some 8% in lactate. Since the water space is likely to be larger than the lactate space, it appears that 90% or so of the injected dose has been detritiated by 5 min. With ^{14}C at 5 min after injection lactate accounts for only 15–20% of the total radioactivity in plasma.

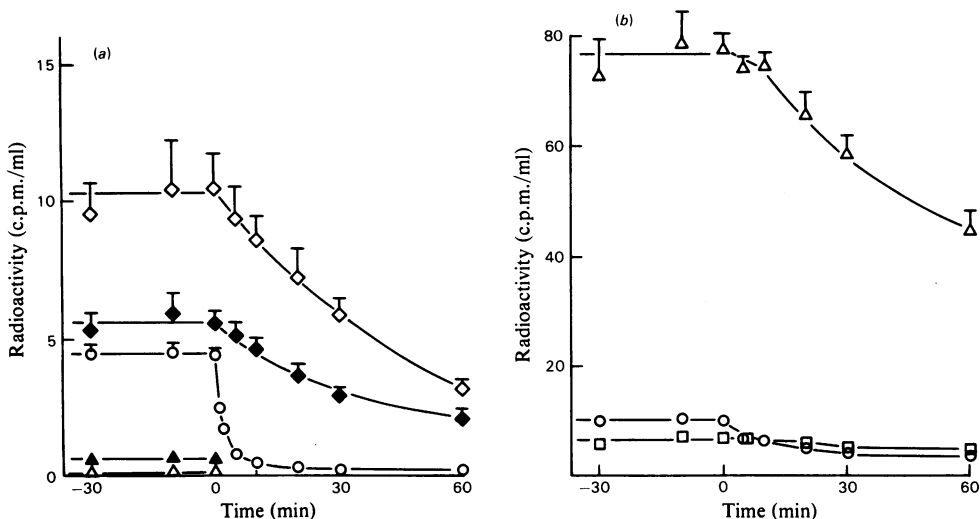


Fig. 3. Radioactivity in plasma in infusion of labelled lactate and wash-out in the A–VC mode

Data are from the experiments of Fig. 2, but the results are expressed as c.p.m./ml of plasma. (a) $[2\text{-}^3\text{H}]\text{-}$ Lactate; (b) $[U\text{-}^{14}\text{C}]\text{-lactate}$. (a) Radioactivity in: O, $[^3\text{H}]\text{lactate}$; ◇, glucose from $[3\text{-}^3\text{H}]\text{lactate}$; ◆, glucose from $[2\text{-}^3\text{H}]\text{lactate}$; ▲, amino acids from $[3\text{-}^3\text{H}]\text{lactate}$; △, amino acids from $[2\text{-}^3\text{H}]\text{lactate}$. (b) Radioactivity in: O, $[U\text{-}^{14}\text{C}]\text{lactate}$; △, glucose from $[U\text{-}^{14}\text{C}]\text{lactate}$; □, amino acids from $[U\text{-}^{14}\text{C}]\text{lactate}$.

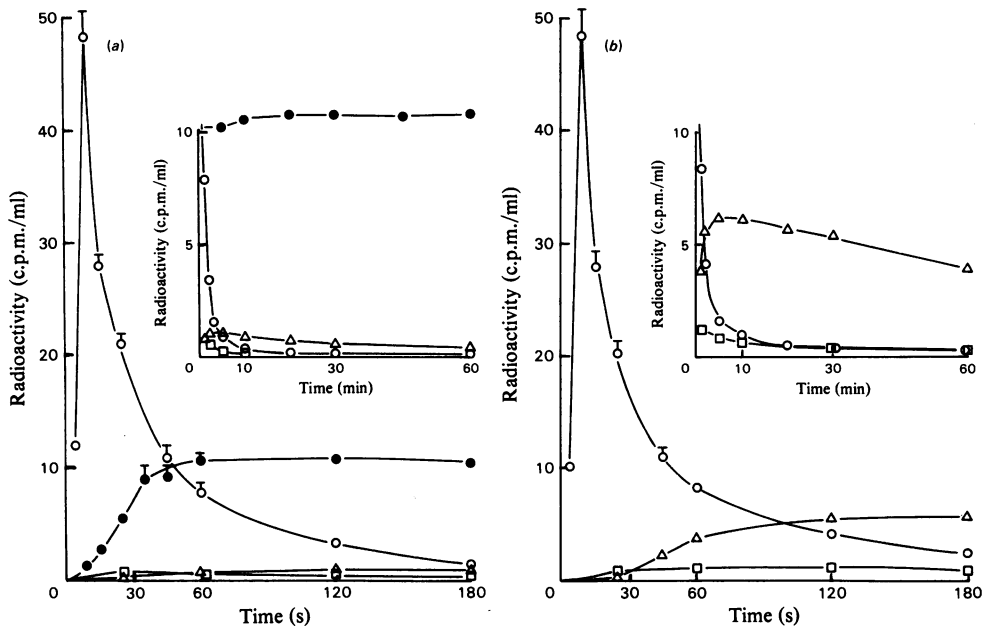


Fig. 4. Radioactivity in plasma after injection of $[3\text{-}^3\text{H},\text{U}\text{-}^{14}\text{C}]$ lactate

$[3\text{-}^3\text{H},\text{U}\text{-}^{14}\text{C}]$ Lactate was injected either into the aorta and sampled from the vena cava or injected and sampled from the aorta (see the text). Results are normalized to an injected dose of 10000 c.p.m./kg body wt., and expressed as c.p.m./ml of plasma. (a) and inset, $[3\text{-}^3\text{H}]$ Lactate. (b) and inset, $[\text{U}\text{-}^{14}\text{C}]$ Lactate. Radioactivity in: O, lactate; Δ, glucose; □, amino acids; ●, water.

Replacement rate

We summarize below our data for the calculations of the apparent replacement rates of lactate obtained in the A-VC and V-A modes. The conventional method to calculate replacement rate for continuous infusion (the apparent replacement rate, R , equals infusion rate/plateau specific radioactivity) and for bolus injection (R equals injected dose/area under the specific-radioactivity curve) are used. We present throughout the observed range, the mean and the standard error of the mean.

In Table 1 the average values and ranges for plateaus of lactate and glucose obtained in continuous infusion in the A-VC and V-A modes are presented. In the A-VC mode the specific radioactivity of $[2\text{-}^3\text{H}]$ lactate was somewhat higher than that of $[3\text{-}^3\text{H}]$ lactate (70 ± 5 versus 63 ± 7 c.p.m./mg), and there was virtually no difference in the V-A mode. The difference in the A-VC mode was statistically not significant, and we have combined for each mode the lactate specific-radioactivity curves of the two ^3H -labelled tracers. The mean replacement rate for $[^3\text{H}]$ lactate was 15.2 mg/min per kg body wt. in the A-VC mode, much greater than that in the V-A mode, which was 8.7 mg/min per kg body wt. For $[\text{U}\text{-}^{14}\text{C}]$ lactate the corresponding rates were 7.4 and 5.6 mg/min per kg body wt.

Similar values for the replacement rates

(16.9 ± 2.9 mg/min per kg body wt. for $[2\text{-}^3\text{H}]$ lactate, 7.0 ± 1.2 mg/min per kg body wt. for $[\text{U}\text{-}^{14}\text{C}]$ lactate) were obtained in experiments when infusion of labelled lactate was in the descending aorta downstream from the mesenteric and renal arteries and sampling was from the aorta (A-A mode; see Katz *et al.*, 1981).

The results with single injection are summarized in Table 2. Of the six experiments listed in the A-VC mode, three were performed by injecting into the aorta and sampling from the vena cava, and three experiments were performed by injecting into and sampling from the aorta (see the Methods section). Sampling was commenced some 3 s after the injection of labelled lactate. The time of maximal specific radioactivity was the same, and the curves for the two procedures were indistinguishable. For single injection this procedure has the advantages of having a single cannula, and permitting rapid sampling.

Measuring of the areas is difficult, especially in the V-A mode. Maximal specific radioactivities occur very early, the decay is extremely rapid and with ^3H -labelled lactate three-quarters or more of the total area is subtended in the first 2 min after injection (see Katz *et al.*, 1981). It is, in practice, virtually impossible to fit the data to exponential expressions. A similar situation was encountered by Kallai-Sanfacon *et al.* (1978) in their study of the

Table 1. *Infusion of labelled lactate*

[2-³H,U-¹⁴C]Lactate and [3-³H,U-¹⁴C]lactate were infused with priming in either the A-VC or the V-A mode (see the text). Specific radioactivities (S.A.) are normalized to an infused dose of 1000 c.p.m./min per kg body wt. Apparent replacement rates were obtained from the ratio of infused rate to the plateau specific radioactivities. Ranges (in parentheses) and means \pm S.E.M. ($n = 9$ for A-VC mode, $n = 10$ for V-A mode) are reported.

Tracer ...	A-VC mode		V-A mode	
	[³ H]Lactate	[U- ¹⁴ C]Lactate	[³ H]Lactate	[U- ¹⁴ C]Lactate
Concn. of plasma lactate (mg/100 ml)	(5.6-11.7)		(6.4-10)	
	8.7 ± 0.8		8.5 ± 0.7	
S.A. of lactate (c.p.m./mg)	(46-81)	(119-176)	(86-155)	(151-241)
	66 ± 4	139 ± 8	120 ± 7	183 ± 10
Replacement rate, <i>R</i> (mg/min per kg)	(12-22)	(6.2-8.8)	(6.5-10.4)	(4.5-6.4)
	15.2 ± 1.2	7.4 ± 0.4	8.7 ± 0.5	5.6 ± 0.3
Plasma lactate clearance (ml/min per kg)	(135-238)	(65-123)	(83-146)	(52-87)
	185 ± 11	87 ± 7	106 ± 5	67 ± 3
S.A. of glucose (c.p.m./mg)				
From [2- ³ H]lactate	(8-14)	—	(10-12)	—
	10 ± 1.3		11 ± 0.3	
From [3- ³ H]lactate	(4-8)	—	(6-9)	—
	5.0 ± 0.5		8.0 ± 0.5	
From [U- ¹⁴ C]lactate		(69-91)		(76-91)
		77 ± 3		82 ± 2
[³ H]Lactate/[¹⁴ C]lactate S.A. ratio		0.47		0.66
Glucose/lactate S.A. ratio				
From [2- ³ H]lactate	0.15		0.09	
From [3- ³ H]lactate	0.09		0.07	
From [U- ¹⁴ C]lactate		0.55		0.45

Table 2. *Injection of [2-³H,U-¹⁴C]lactate and [3-³H,U-¹⁴C]lactate in the A-VC and V-A modes*

Specific radioactivities are normalized to an injected dose of 10000 c.p.m./kg body wt. Ranges (in parentheses) and means \pm S.E.M. ($n = 6$) are reported.

Label in lactate ...	³ H		U- ¹⁴ C	
	A-VC mode	V-A mode	A-VC mode	V-A mode
Lactate curve t_{max} (S)	(9-15)	(3-5)	(9-15)	(3-5)
Fraction of area (%) under curve in period				
0- t_{max}	10	17	4	10
t_{max} - 1 min	41	52	18	33
1 min-20 min	41	27	61	44
20 min- ∞	8	4	17	9
Total area (min·c.p.m./mg)	(450-690)	(940-1340)	(1060-1410)	(1640-2090)
	524 ± 41	1210 ± 73	1180 ± 62	1790 ± 73
Replacement rate, <i>R</i> (mg/min per kg)	(14-22)	(7.5-10.6)	(7.1-9.4)	(4.8-6.0)
	19 ± 1.1	8.5 ± 0.5	8.5 ± 0.4	5.6 ± 0.2
Area for glucose (min·c.p.m./mg)	—	—	580-1110	750-840
			811 ± 58	813 ± 19
[³ H]Lactate area / [¹⁴ C]Lactate area ratio	0.44	0.67		
[¹⁴ C]Glucose area / [¹⁴ C]Lactate area ratio			0.68	0.45

metabolism of [2-³H]glycerol in dog in the V-A mode. They observed, however, a linear relationship in the range 0.25–100 min when the logarithm of specific radioactivity was plotted against logarithm

of time, so that their data could be fitted by a simple power function of the form $S.A. = At^{-\alpha}$, where S.A. represents specific radioactivity. If t is expressed in minutes, the constant A is the specific radioactivity at 1 min. This function approaches infinity as t goes to zero, and hence, for integration, the time at which maximal radioactivity is attained ($t_{max.}$) has to be determined by rapid sampling. The area between time 0 and $t_{max.}$ was obtained graphically (as a triangle with a base $t_{max.}$ and height $S.A._{max.}$), and the rest of the area by integration. The integrated equation is:

$$t_{max.} \cdot S.A. = \frac{A}{\alpha - 1} (t_{max.}^{-\alpha} - t^{-\alpha})$$

We found that the approach of Kallai-Sanfacon *et al.* (1978) was applicable to our results in the V-A mode. In Fig. 5 we present the results of double-logarithmic plots of our data. There was linearity for [³H]lactate from 0.1 up to 60 min, with the function $S.A. = 140t^{-1.29}$. The curve for [U-¹⁴C]lactate coincided for the first 1 min with that for [³H]lactate, and beyond that was approximated by the function $S.A. = 140t^{-0.85}$. Beyond 60 min the specific radioactivities deviated from the logarithmic curve. $t_{max.}$ in arterial blood was determined in a series of experiments and ranged from 3 to 5 s. We thus determined the area by dividing it into three segments. The area from 0 to $t_{max.}$ was measured as a triangle, that from $t_{max.}$ to 60 min by integrating the power function, and the tail end beyond 60 min was estimated by fitting a mono-exponential function and integration to infinity. In the A-VC mode the fit to

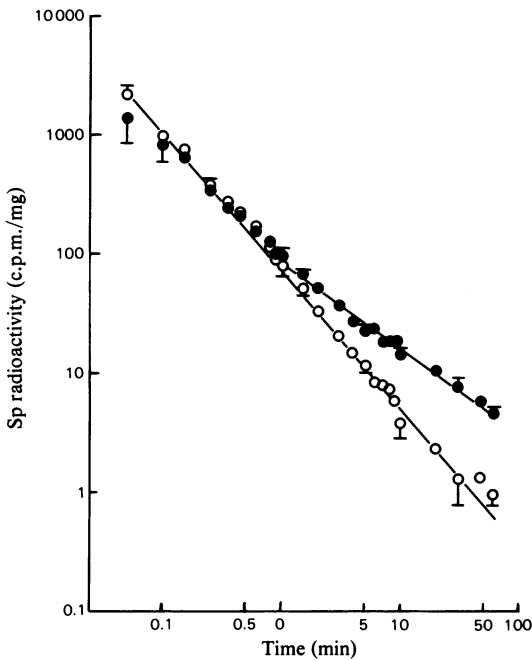


Fig. 5. Logarithmic plot of lactate specific radioactivity in blood
For explanation see the text. O, [³H]Lactate; ●, [U-¹⁴C]lactate.

Table 3. Areas of wash-out curves of lactate and value of mean transit time and lactate mass

Data are from the experiments of Table 1. A, Original experimental results; B, results corrected for recycling of ³H (see the text). Ranges (in parentheses) and means ± s.e.m. ($n = 9$ for A-VC mode, $n = 10$ for V-A mode) are reported.

Tracer ...	[³ H]Lactate				[U- ¹⁴ C]Lactate	
	A-VC mode		V-A mode		A-VC mode	V-A mode
	A	B	A	B	A	A
Plateau (c.p.m./mg)	66 ± 4.4	62 ± 4.4	120 ± 7.0	116 ± 7.5	139 ± 8	183 ± 10
Replacement rate, R (mg/min per kg)	15.2 ± 1.2	16.1 ± 1.2	8.7 ± 0.5	9.0 ± 0.6	7.4 ± 0.4	5.6 ± 0.3
Area (min · c.p.m./mg)	(187–373) 289 ± 25	(96–265) 180 ± 21	(297–524) 395 ± 24	(164–366) 268 ± 21	(6900–11000) 9300 ± 660	(7800–13800) 10100 ± 560
Mean transit time (min)	(3.7–5.6) 4.4 ± 0.2	(2.0–3.8) 3.0 ± 0.2	(2.3–4.0) 3.3 ± 0.2	(1.6–2.9) 2.3 ± 0.15	(50–88) 67 ± 4	(46–73) 56 ± 2.5
Mass (mg/kg)	(53–97) 69 ± 5.6	(33–62) 48 ± 3.7	(17–38) 28 ± 2.3	(12–28) 20 ± 2.2	(350–740) 497 ± 40	(220–440) 317 ± 27
Concn. of plasma lactate (mg/100 ml)	8.7 ± 0.8		8.5 ± 0.7			
Lactate space (ml/kg)	(660–1380) 882 ± 98	(410–900) 662 ± 62	(262–410) 339 ± 31	(170–290) 241 ± 25		

the power function was less satisfactory, and it was practical to obtain the area up to 20 min by graphical means (cutting out and weighing paper). The tail area beyond 20 min was estimated by integration to infinity of a mono-exponential function. In Table 2 we present the fraction of total area measured in the different time intervals. In the V-A mode 69% of the area for ^3H lactate and 43% of that for $[\text{U-}^{14}\text{C}]$ lactate was accounted for in the first 1 min. In the A-VC mode the corresponding fractions for ^3H lactate and $[\text{U-}^{14}\text{C}]$ lactate were 51 and 22%.

The apparent replacement rates for the A-VC mode were 19 and 8.5 mg/min per kg body wt. for ^3H lactate and $[\text{U-}^{14}\text{C}]$ lactate respectively, and in the V-A mode 8.5 and 5.6 mg/min per kg body wt. respectively. The agreement between the results obtained by infusion and injection were well within the experimental error of these determinations. Similar values for replacement, 17 mg/min per kg body wt., were obtained when tracer was infused into the descending aorta, distal to the expanding of the mesenteric and renal arteries (see below and Table 4). Sampling was from the aortic arch. This procedure, designated the A-A mode, differs from the A-VC mode primarily in that the first passage of the tracer does not include the splanchnic bed and liver and kidneys, the sites of glucose synthesis.

As is apparent from Tables 1 and 2, there was a considerable variability between animals in lactate concentration and in the rate of replacement. We noted that animals with the highest plasma lactate concentrations showed high replacement rates. In Fig. 6 we show the correlation of lactate concentrations and apparent replacement rates in infusion in the A-VC and A-A modes for ^3H lactate and $[\text{U-}^{14}\text{C}]$ lactate. The correlation coefficient for ^3H lactate $r=0.92$ and that for $[\text{U-}^{14}\text{C}]$ lactate $r=0.81$, showing a highly significant correlation between plasma lactate concentration and apparent replacement rate. A high correlation between lactate turnover and concentration was shown by Freminet *et al.* (1974). The apparent rates of lactate clearance were very high, 180 and 90 ml/min per kg body wt. for ^3H lactate

and $[\text{U-}^{14}\text{C}]$ lactate respectively in the A-VC mode. The corresponding clearance rates in the V-A mode were 106 and 67 ml/min per kg body wt.

Blood lactate concentrations may vary over a very wide range. In vigorous exercise in man, transient plasma and muscle lactate concentrations as high as 25 mM are attained. In our experiments with starved rats the lactate concentrations ranged from about 0.5 to 1.5 mM. Plasma lactate concentrations were more labile than those of glucose. In experiments where sampling was simultaneously from two sites, the lactate concentration in plasma frequently increased markedly up to 3 mM, probably owing to stress. Although lactate concentrations may vary, it appears that the fractional rate of lactate clearance is quite stable for the concentration range of our experiments.

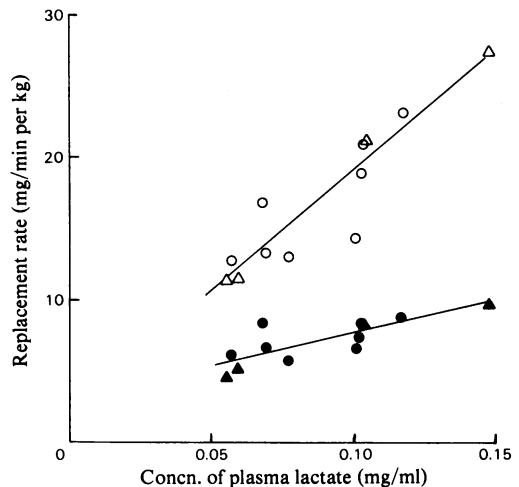


Fig. 6. Linear-regression plot of lactate replacement rate versus concentration

Experiments were performed with continuous infusion in the A-VC mode. ● and ○, Lactate infused into the aortic arch and sampled from the vena cava; ▲ and △, lactate infused into the descending aorta arch. ● and ▲, $[\text{U-}^{14}\text{C}]$ Lactate; ○ and △, $[\text{U-}^{14}\text{C}]$ and $[\text{U-}^{14}\text{C}]$ -lactate.

Table 4. Replacement rates and lactate mass in the A-A mode

Primed infusion with $[\text{U-}^{14}\text{C}]$ lactate in the A-A mode (see the text) was used. Tracer was infused after priming in the descending aorta and sampling was from the aortic arch. The mean transit time for $[\text{U-}^{14}\text{C}]$ lactate was corrected for recycling of ^3H . Ranges (in parentheses) and means \pm s.e.m. ($n=4$) are reported.

Tracer ...	^3H Lactate	$[\text{U-}^{14}\text{C}]$ Lactate
Concn. of plasma lactate (mg/100 ml)	(5.9-15) 9.1 ± 2.2	(5.2-9.8) 7.0 ± 1.2
Replacement rate, R (mg/min per kg)	(11-26) 16.9 ± 2.9	(79-88) 64 ± 8.6
Mean transit time (min)	(3.1-6.1) 4.3 ± 0.7	(335-535) 420 ± 42
Mass (mg/kg)	(36-111) 73 ± 15	—
Lactate space (mg/kg)	(610-1280) 848 ± 148	—

Recycling of lactate carbon and the contribution of lactate carbon to glucose

The apparent replacement rates (averaging continuous infusion and injection) for ^3H and ^{14}C in the A-VC mode are 17 and 8 mg/min per kg body wt. respectively, and hence the recycling of lactate carbon in this mode is $100 \times (17-8)/17 = 53\%$. The corresponding value for the V-A mode is $100 \times (8.6-5.6)/8.6 = 35\%$. This method for calculation of recycling of glucose carbon was introduced by Katz *et al.* (1974a) for glucose. It was widely adopted as a method for the calculation of the Cori cycle. This is not correct (see Katz *et al.*, 1976, 1979). Recycling of glucose carbon is not the rate of reconversion of lactate into glucose. To illustrate this, assume that 100% of catabolized glucose is converted into lactate, and this *in toto* is reconverted into glucose (100% Cori cycle). However, owing to influx or exchange of carbon from other sources into oxaloacetate the $^3\text{H}/^{14}\text{C}$ ratio and recycling will be less than 100%. However, it is likely (for most cases) that the greater the operation of the Cori cycle the greater the fraction of carbon recycled.

The contribution of lactate carbon to glucose carbon may be obtained from the ratios of specific radioactivities at isotopic steady state (Table 2) or from the ratios of areas under the respective specific-radioactivity curves (Table 3). The ratios in continuous infusion did not differ too greatly for the two modes, being 0.55 in the A-VC mode and 0.45 in the conventional V-A mode. The same ratio (0.45) was obtained from single injection in the V-A mode, but a higher ratio (0.67) for the A-VC mode (Table 2).

It is well known that in the conversion of ^{14}C lactate into glucose there is a substantial dilution of the carbon of oxaloacetate and phosphoenolpyruvate by dilution with carbon influx from the tricarboxylic acid cycle (see review by Katz, 1979). In isolated hepatocytes from starved rats the rates of gluconeogenesis from lactate obtained by direct analysis are 30–50% higher than those calculated from isotopic yields in glucose. Hence the correction for dilution is by a factor of 1.3–1.5. By applying the same correction *in vivo* to the A-VC mode we estimate that lactate contribution to gluconeogenesis is in the range 70–85%. Even for the V-A mode the lactate appears to be the precursor of 60–70% of newly synthesized glucose.

Virtually all other workers, using the V-A mode, obtained much lower estimates for the contribution of lactate carbon to glucose than in our studies. For example, 10% (Depocas *et al.*, 1969) and 17% (Forbath & Hetenyi, 1970) in dogs and 17% in rats (Freminet & Poyart, 1975) have been reported. It is possible that in continuous infusion there was frequently a failure to attain isotopic steady state

because it was not realized that the terminal slope of the ^{14}C lactate specific-radioactivity curve is small.

Incorporation of ^3H into glucose

The yield of tracer in glucose was much the same in the A-VC mode as in the V-A mode, but the ratios of specific radioactivity at isotopic steady state of glucose to lactate were higher in the A-VC mode. In this mode the ratios were 0.55 for $[\text{U-}^{14}\text{C}]\text{lactate}$, 0.15 for $[\text{2-}^3\text{H}]\text{lactate}$ and 0.09 for $[\text{3-}^3\text{H}]\text{lactate}$ (Table 1). ^3H from $[\text{2-}^3\text{H}]\text{lactate}$ is incorporated into glucose via reduction with NAD^3H , formed in the lactate dehydrogenase reaction, whereas the major path of ^3H incorporation from $[\text{3-}^3\text{H}]\text{lactate}$ is carbon-bound into phosphoenolpyruvate. A dilution of the NAD^3H pool by other sources of reducing equivalents may have been expected, so that the above results were unexpected. However, Rognstad & Wals (1976) observed in hamster hepatocytes *in vitro* a preferential ^3H incorporation into glucose from $[\text{2-}^3\text{H}]\text{lactate}$ over that from $[\text{3-}^3\text{H}]\text{lactate}$. They found $^3\text{H}/^{14}\text{C}$ ratios in glucose from $[\text{2-}^3\text{H}, \text{U-}^{14}\text{C}]\text{lactate}$ (as compared with the ratio of 1.0 in substrate lactate) to range from 0.3 to 0.4 from $[\text{2-}^3\text{H}, \text{U-}^{14}\text{C}]\text{lactate}$ and from 0.1 to 0.15 from $[\text{3-}^3\text{H}, \text{U-}^{14}\text{C}]\text{lactate}$. Rognstad & Wals (1976) attributed this to the loss of ^3H from the $[\text{3-}^3\text{H}]\text{pyruvate}$ by the reversible transaminase reaction. The labilization of ^3H in this reaction was described by Oshima & Tamiya (1961). Recycling between pyruvate and phosphoenolpyruvate would also account for the lowered ^3H yield in glucose. Since after injection of $[\text{6-}^3\text{H}, \text{6-}^{14}\text{C}]\text{glucose}$ in rats the $^3\text{H}/^{14}\text{C}$ ratio in lactate is much the same as that in circulating glucose (Dunn *et al.*, 1968; see also below), it appears that the detritiation of $[\text{3-}^3\text{H}]\text{lactate}$ occurs only within the cytosol of liver cells.

In several experiments the glucose formed from $[\text{2-}^3\text{H}]\text{lactate}$ and $[\text{3-}^3\text{H}]\text{lactate}$ was isolated and degraded. The results are summarized in Table 5. ^3H from $[\text{2-}^3\text{H}]\text{lactate}$ was mainly in position 4 and to a smaller extent in position 6 of glucose. ^3H from $[\text{3-}^3\text{H}]\text{lactate}$ appeared mainly in position 6, about twice as much as in position 1, and was also present in position 4 of glucose. The results are consistent with the stereospecific pathways of incorporation outlined by Hoberman (1958) and Rose *et al.* (1969). A brief outline of the pathways to account for the incorporation pattern are now presented.

^3H from $[\text{2-}^3\text{H}]\text{lactate}$ labels NAD^3H . In the reduction of 1,3-bisphosphoglycerate and the subsequent isomerization of triose phosphates, ^3H is incorporated on C-1 of glyceraldehyde 3-phosphate and stereospecifically in one position on C-1 of dihydroxyacetone phosphate. The ^3H from dihydroxyacetone is removed in the aldolase-catalysed condensation to fructose 1,6-bisphos-

Table 5. *Degradation of glucose synthesized from [2- ^3H]lactate or from [3- ^3H]lactate*

In experiments with rats 1–6 the ^3H on C-1 and C-6 was determined, and that on C-4 was calculated by difference. In experiments with rats 7 and 8, the ^3H on C-4 was obtained by a direct method (see the text).

Rat no.	Tracer	Tracer administration	Time of sampling	Distribution of ^3H (%)		
				C-1	C-4	C-6
1	[2- ^3H]Lactate	Infusion	Steady state	5.7	60	34
			Wash-out, 20 min	5.1	63	32
2	[2- ^3H]Lactate	Infusion	Steady state	2.0	66	32
3	[2- ^3H]Lactate	Injection	5 min	3.7	64	32
			20 min	2.9	65	32
Mean	[2- ^3H]Lactate			4.0	64	32
4	[3- ^3H]Lactate	Infusion	Steady state	27	20	53
5	[3- ^3H]Lactate	Infusion	Steady state	19	27	53
6	[3- ^3H]Lactate	Infusion	Steady state	17	15	68
7	[3- ^3H]Lactate	Injection		12	29	59
8	[3- ^3H]Lactate	Injection		12	27	61
Mean	[3- ^3H]Lactate			17	24	59

phate, so that ^3H is retained on C-4 of hexose phosphates and glucose. In a second pathway, via the reduction by oxaloacetate in the reversible malate dehydrogenase reaction followed by the reversible dehydration to fumarate, ^3H appears on C-2 and C-3 of fumarate. By the reverse of this reaction sequence ^3H labels stereospecifically one of the hydrogen atoms on C-3 of oxaloacetate and on C-3 of phosphoenolpyruvate, appearing on C-6 and C-1 of fructose 6-phosphate. However, in the isomerization to glucose 6-phosphate the ^3H on C-1 is stereospecifically lost, so that ^3H appears only on C-6 of glucose.

^3H from [3- ^3H]pyruvate labels both hydrogen atoms on C-3 of phosphoenolpyruvate and on C-1 and C-6 of fructose 1,6-bisphosphate. Half of the ^3H from C-1 is lost in the isomerization of fructose 6-phosphate into glucose 6-phosphate, so that the ^3H yield on C-6 and C-1 of glucose should be 2:1. Also, ^3H from oxaloacetate, via the set of reversible reactions described above, labels stereospecifically the hydrogen atom on C-3 of malate. This is transferred to NADH in the malate dehydrogenase reactions, and by pathways described above will introduce ^3H mainly into position 4 and to a smaller extent into position 6 of glucose. We found somewhat less ^3H in position 1 of glucose from [3- ^3H]lactate, as was expected from this scheme, but otherwise the results of Table 5 are in the main consistent with the theory.

Recycling of ^3H in lactate

In [^3H]glucose turnover the total yield of ^3H in labelled compounds in plasma is low and recycling of ^3H into glucose is of negligible significance. With [^3H]lactate the situation is different. Blood glucose concentration normally greatly exceeds that of

lactate, and total radioactivity of ^3H in plasma glucose equals or exceeds that in plasma lactate. When infusion of glucose was stopped, the decay of [^3H]lactate specific-radioactivity curves is much more rapid than that of glucose specific radioactivity, and as shown in Fig. 3(a), after several minutes the specific radioactivity of tritiated glucose exceeded that of lactate. Thus it appeared probable that ^3H recycling would have a significant effect on the terminal slope of the lactate specific-radioactivity curves, and would have to be considered in the estimation of mean transit time and lactate mass.

The recycling of ^3H between glucose and lactate would depend mainly on in what positions glucose is labelled by lactate. As shown in Table 5, these positions are 6, 1 and 4. To determine the incorporation of ^3H from glucose into lactate, starved rats were injected with [6- ^3H]glucose and [4- ^3H]glucose. Dunn *et al.* (1968) obtained, after injection of [6- ^3H ,6- ^{14}C]glucose, $^3\text{H}/^{14}\text{C}$ ratios in lactate that were equal to or somewhat higher than that in glucose. In the present work we obtained from [6- ^3H ,U- ^{14}C]glucose $^3\text{H}/^{14}\text{C}$ ratios of 0.8 in lactate (relative to glucose), and the specific radioactivity of ^3H -labelled lactate at isotopic steady state ranged from 50 to 70% of that in circulating glucose. The specific radioactivities of lactate from [4- ^3H]glucose ranged from 10 to 20% of those of glucose.

The correction factors for recycling were calculated by assuming incorporation of 60% of ^3H from positions 6 and 1 of glucose into lactate and 15% from position 4 and by using the distribution of ^3H in glucose from Table 5. The correction factors are approximately 0.5 for [3- ^3H]lactate and 0.33 for [2- ^3H]lactate. The specific radioactivities of glucose were multiplied by the appropriate factor and subtracted from the specific radioactivities of lactate.

Our experimental results are too limited to justify a more rigorous procedure such as deconvolution, and these are only rough but probably adequate approximations.

The specific radioactivity of [^3H]lactate corrected for recycling is shown by the broken line in Fig. 2. The correction is of the order of 5% for the specific radioactivity at isotopic steady state, but the corrections have a highly significant effect on the areas under the wash-out curves and their terminal slopes. In bolus injection corrections for recycling decrease the terminal slope (tail) of the lactate specific-radioactivity curves, but have a negligible effect on the area under the curve. The areas under the wash-out curve provide an estimate of mean transit time and total body lactate mass, and, as shown in the next section, recycling of ^3H has a marked effect on these calculations.

Mean transit time and mass

Mean transit time, \bar{t} , and total lactate mass (the product of \bar{t} and the replacement rate R) can be calculated graphically only for a model with injection and sampling from the inlet-outlet compartment of a mamillary system, or in a non-compartmental system, when sampling is from the single outlet (see Katz *et al.*, 1981). The simplest method is from the wash-out curve after continuous infusion, since:

$$\bar{t} = \frac{\text{Area under wash-out curve}}{\text{Plateau specific radioactivity}}$$

The area must be evaluated from the time infusion is stopped to infinity. In Table 3 we present the areas of the wash-out curves in both modes, without and with corrections for recycling. The area was obtained graphically for the segment up to 20 min, and by exponential extrapolation beyond 20 min. In the corrected curves the latter segment accounted for only a few percent of total area.

The corrections for recycling increased the replacement rates by about 5%, but they decreased the areas of the wash-out curves by 30–40% (Table 3). The corrected value for mean transit time in the A–VC mode was about 3 min, yielding a total body lactate mass of 48 mg/kg body wt. In the V–A mode the apparent mean transit time was smaller, mainly because of the higher plateau specific radioactivity, and calculated apparent mass was, after correction for recycling, 20 mg/kg body wt. The lactate concentration in plasma ranged quite widely in our experiments, averaging for all experiments about 0.085 mg/ml of plasma. Thus the 'lactate space' in the A–VC mode averages to a value some 66% of body weight but only 24% for the V–A mode. However, variability was high, with the range for nine experiments for the A–VC mode being from 41 to

90% of body space. The percentage body water in all our experiments with single injection was calculated from the final ^3HOH content of plasma. It ranged from 79 to 91%, with an average of about 85%. Similar results were observed by us previously for overnight-starved lean rats (Katz *et al.*, 1979). This water includes also that in the gut and bladder.

A higher mean transit time, 4.3 ± 0.7 min, was obtained when tracer was infused into the descending aorta (A–A mode) (Table 4), corresponding to a lactate mass of 73 mg and a lactate space of 85%. The number of experiments in these modes was limited (four rats), and lactate concentration and mass were quite variable (from 36 to 111 mg/kg body wt.), so that it is difficult to assess whether the difference in transit time between the A–VC and A–A modes is significant.

The estimation of mean transit time from curves in single injection requires the evaluation of the integral $\int_0^{\infty} S.A._{(t)} \cdot t \cdot dt$ (Katz *et al.*, 1974a). The evaluation of this integral is very sensitive to the values of the specific radioactivities when t becomes large. The evaluation of the terminal slope is experimentally rather difficult and subject to considerable error. Evaluation of the integral, not presented here in detail, gave average values in reasonable agreement with those of Table 3, but the variability and standard error were large, and the results are not presented here.

In Table 3 we present also the apparent mean transit times for [$\text{U-}^{14}\text{C}$]lactate in the two modes. The transit times were of the order of 1 h, greater than those calculated for glucose (Katz *et al.*, 1974b). The apparent mass of lactate carbon of the A–VC mode ranged from 350 to 740 mg/kg body wt. (mean about 500 mg/kg body wt.). This must include not only the mass of carbon of lactate, pyruvate and alanine, which are likely to equilibrate readily, but that of glucose. The total mass of these carbon sources is not likely to exceed 350 or at most 400 mg/kg body wt. It may include the same carbon in glutamate, glutamine and some rapidly turning-over proteins, but this remains to be established.

Discussion

^3H and ^{14}C as tracers for lactate

The merit of ^3H as a tracer is that it may serve as an irreversible label, which is not re-incorporated back into the mother compound (see Katz *et al.*, 1974a). Such a tracer would be especially valuable for lactate, where recycling of carbon is very extensive. The use of ^3H may, however, pose problems with some types of labelling. It was realized with [$2\text{-}^3\text{H}$]glucose that detritiation may be in part due to futile cycling in liver, so that ^3H loss, though a measure of glucose phosphorylation, overestimates net glucose uptake (Katz, 1979). With

lactate there is no evidence for futile cycling, which involves energy dissipation, but detritiation by exchange with protons is a theoretical possibility.

If the exchange between lactate and pyruvate is very rapid, and if cytosolic NAD^3H derived from $[2\text{-}^3\text{H}]\text{lactate}$ were diluted by unlabelled reducing equivalents, in effect an exchange of carbon-bound ^3H occurs and detritiation would exceed net lactate utilization. There is no experimental evidence for this exchange. With $[3\text{-}^3\text{H}]\text{lactate}$, a $^3\text{H}/^1\text{H}$ exchange from $[3\text{-}^3\text{H}]\text{pyruvate}$ by alanine aminotransferase, coupled with rapid equilibration of pyruvate and lactate, may also release ^3H in excess of net pyruvate utilization. In hamster hepatocytes Rognstad & Wals (1976) observed a more rapid utilization of $[3\text{-}^3\text{H}]\text{lactate}$ than that of $[\text{U-}^{14}\text{C}]\text{lactate}$, whereas uptake of $[2\text{-}^3\text{H}]\text{lactate}$ and $[\text{U-}^{14}\text{C}]\text{lactate}$ were equal. Since in the presence of cycloserine, an aminotransferase inhibitor, uptake of $[3\text{-}^3\text{H}]\text{lactate}$ and $[\text{U-}^{14}\text{C}]\text{lactate}$ became equal, excess detritiation was believed to be due to aminotransferase-catalysed exchange at the pyruvate level.

In vivo, however, Dunn *et al.* (1968) observed after $[6\text{-}^3\text{H}, \text{U-}^{14}\text{C}]\text{glucose}$ injection much the same $^3\text{H}/^{14}\text{C}$ ratio in glucose as in lactate. We also found only a small decrease in the $^3\text{H}/^{14}\text{C}$ ratio in lactate from $[6\text{-}^3\text{H}, \text{U-}^{14}\text{C}]\text{glucose}$. This indicates that *in vivo* transamination has little effect on the determination of lactate turnover. The results obtained by Rognstad & Wals (1976) show that dilution by ^3H exchange at the NADH level does not occur in liver. It appears also unlikely that dilution of cytosolic NADH from lactate by exchange with mitochondrial NADH would occur in muscle. The fact that the kinetics of $[3\text{-}^3\text{H}]\text{lactate}$ and $[2\text{-}^3\text{H}]\text{lactate}$ are nearly the same suggests that detritiation is a valid measure of lactate utilization. Detritiation catalysed mainly by aminotransferase (see above) occurs in the interconversion $\text{lactate} \rightarrow \text{alanine} \rightarrow \text{lactate}$, but it is not established to what extent this occurs. Equilibrium between lactate and alanine is incomplete, and the specific radioactivities and kinetics of these compounds differ considerably (J. Katz, unpublished work). At the present time caution in the interpretation of the physiological significance of the turnover of $[^3\text{H}]\text{lactate}$ is called for, and further studies are required.

Describing the kinetics of lactate turnover

The A-VC and V-A modes constitute injection or sampling at different sites of the same system. Ideally, with a suitable analysis the curves for the two modes should be consistent and yield the same values for replacement and mass and other parameters of lactate metabolism. For analysing the data or computer modelling it is advantageous to describe the respective curves by some functions,

most conveniently exponential expressions. The most reliable procedure to obtain the characteristics of the curves is from the wash-out curves. The exponents are the same for bolus injection or infusion, and the coefficients in injection (A values) are related for unit dose to those in wash-out or unprimed infusion (B values) by eqn. (1) (see Katz *et al.*, 1974a):

$$A_i/\alpha_i = B_i \quad (1)$$

As an illustration, on infusing 1000 c.p.m./min per kg body wt. a wash-out curve

$$\text{S.A.} = 60e^{-1.2t} + 40e^{-0.12t}$$

is obtained. If 10000 c.p.m./kg body wt. were injected the coefficients would be:

$$60 \times 1.2 \times \frac{10000}{1000} = 720$$

and

$$40 \times 0.12 \times \frac{10000}{1000} = 48$$

Normalizing so that the specific radioactivity at plateau or zero-time intercept is unity, the two curves would have the same exponents, and coefficients of 0.6 and 0.04 for the unprimed infusion or wash-out curve, and 0.94 and 0.06 for bolus injection. Obviously a reliable experimental estimate of the early and terminal part of the bolus curve would be subject to a much greater error than in the wash-out curve.

In Table 6 we summarize the coefficients and exponents for the wash-out curves for the A-VC and V-A modes. The curves were obtained by manual curve peeling. When the ^3H specific-radioactivity curves were corrected for recycling (see above), the specific radioactivities beyond 30 min became negligible. The wash-out curves for ^3H -labelled and ^{14}C -labelled lactate were best represented by three exponentials, although the mass of carbon was much larger. As estimated from the area of the wash-out curves (Table 3) the mass corresponding to the $[^3\text{H}]\text{lactate}$ specific-radioactivity curve was, whatever the mode, about one-tenth of the mass from the $[\text{U-}^{14}\text{C}]\text{lactate}$ specific-radioactivity curve. An association of the number of exponents to an equal number of discrete pools seems to be untenable, and the kinetic parameters are considered mainly as a means to describe the shape of the curve and for numerical calculations.

In Table 6 we present also the transformed coefficients for bolus injection. The last coefficient is very small, a fraction of a percent of the total sum, and in practice would not be detected in experiments with single injections.

We have used the data of Table 6 in trying to analyse the system in terms of two well-mixed pools,

Table 6. *Coefficients and exponents for wash-out curves for labelled lactate and for bolus injection*

The curve for wash-out curve is $\Sigma B_i e^{-\alpha_i t}$ and that for bolus injection $\Sigma A_i e^{-\alpha_i t}$. Values are normalized to an injection of 1000 c.p.m./kg body wt. and to an infusion of 1000 c.p.m./min per kg body wt. t is in min. The exponents and coefficients were determined for the wash-out curves. The coefficients for injection were calculated (see the text). The exponents are the same for both curves.

Mode	Label in lactate	Correction for recycling	Infusion			α_1	α_2	α_3	Injection		
			B_1	B_2	B_3				A_1	A_2	A_3
A-VC	$U-^{14}C$	No	49.5	28.9	60.2	0.72	0.082	0.007	35.6	2.4	0.42
	3H	No	33.5	25.6	6.9	1.54	0.37	0.032	51.7	9.4	0.22
	3H	Yes	46.4	11.2	4.2	0.98	0.26	0.046	45.2	3.1	0.19
V-A	$U-^{14}C$	No	99.2	20.6	62.9	0.96	0.069	0.0064	95.2	1.4	0.40
	3H	No	85.7	26.7	7.2	1.92	0.30	0.022	165	8.0	0.16
	3H	Yes	89.0	21.7	4.9	1.59	0.28	0.033	172	6.2	0.16

with an inflow and outflow into each (see Steele, 1971), but were unable to obtain consistent and reasonable solutions for both replacement rate and mass. A more complex model may be required, or we may not have well-mixed pools. The problem in applying conventional analysis to the two modes may be of a more fundamental nature. All previous studies of tracer kinetics are based on the implicit assumption that the administered tracer labels uniformly the newly formed tracee. This probably holds for compounds confined mainly to extracellular fluid, such as glucose, plasma proteins or blood-borne hormones. The general requirement is that, if the tracee enters the system at various sites, there must be 'equivalent labelling' (Steele, 1971) proportional to the input of tracee at each site of synthesis. If there is no 'equivalent labelling', there are to our knowledge no appropriate means for analysing tracer kinetics from specific radioactivities in blood. Lactate is formed in many tissues, much or most of it is intracellular, and, though there is no doubt rapid exchange between cell and extracellular lactate, it is quite possible that tracer injection into blood does not assure perfect 'equivalent' labelling of lactate.

A-VC mode versus the V-A mode

In the preceding paper (Katz *et al.*, 1981) we presented a simple model, which accounted qualitatively for kinetics of the two modes. According to that model, conventionally used calculations for replacement rate and mass are valid only for the A-VC mode [eqns. (1) and (2) of Katz *et al.* (1981)]. The model no doubt was over-simplified, and does not account quantitatively for the two modes. Although there is no definite proof of the validity, it is likely that calculations based on the A-VC mode provide more realistic estimates of the parameters of lactate metabolism than do those based on the V-A mode. This is supported by the following observations.

(1) The mass of lactate as calculated from the [3H]lactate specific-radioactivity curve in the A-VC mode is about 50 mg/kg body wt., and the lactate space is about 65% of the body. In the V-A mode the mass is less and the lactate space is 24%, approximately the extracellular water space. It is well established that lactate occurs in cells in about the same concentration as in extracellular fluid, and a lactate space of 65% appears to be a realistic estimate. The lower estimate of the V-A mode is most unlikely.

(2) Lactate turnover is very rapid. Maximal radioactivity in 3H - and ^{14}C -labelled amino acids and in water occurs within 1 min after injection, and near-maximal radioactivity of glucose is attained within 2 min. At that time only a small fraction of injected dose is present as lactate. The replacement rate of [3H]lactate in the A-VC mode ranged from 15 to 18 mg/min per kg body wt. (Tables 1, 2 and 4), as compared with 8 mg/min per kg body wt. in the V-A mode only. The higher rate in the A-VC mode is compatible with such a turnover.

(3) The equilibration between [^{14}C]lactate, [^{14}C]glucose and ^{14}C -labelled amino acids is extensive. The recycling of ^{14}C of lactate is 60% in the A-VC mode and 35% in the V-A mode. The former rate is much more compatible with the data. Within the framework of conventional isotope analysis, it is the A-VC mode that yields a much better approximation of the physiological parameters of the lactate metabolism than do calculations based on the V-A mode.

[3H]Lactate turnover

The rate of lactate replacement, averaging 16 mg/min per kg body wt., exceeds that of glucose, 8-10 mg/min per kg body wt. (Katz *et al.*, 1974b). The question is: what are the major products of lactate metabolism and what are the precursors for lactate synthesis? Glucose is a major product of lactate metabolism in liver, as well as a major

precursor of lactate in extrahepatic tissues. However, glucose synthesis accounts only in part for lactate uptake. It has been established in recent years that lactate is a major precursor of liver glycogen (Katz *et al.*, 1979) and fatty acids (Clark *et al.*, 1974; Bloxham *et al.*, 1977). Lactate is formed by glycolysis in muscle, and it is also secreted into portal blood by the intestinal mucosa. Further studies are required to account for the source of lactate.

Mean transit time and mass

The mean transit time for lactate was obtained from the area of the wash-out curve for [^3H]lactate. The transit time equals the area under the curve divided by plateau specific radioactivity. On theoretical and experimental grounds the estimate is less reliable than that of replacement rate. Also, as we have shown, recycling of ^3H affects significantly the area under the wash-out curve, and requires corrections for recycling, an unstable operation. The mean transit time, corrected for recycling, was about 3 min, or one-third of the lactate is turned over per min.

The specific-radioactivity curve after bolus injection is proportional to a plot of frequency distributions of transit times (Zierler, 1962), and hence the area under the curve, and the (unprimed) infusion or wash-out curves are proportional to the cumulative transit time. For example, if in unprimed infusion the specific radioactivity at 3 min were 67% of the plateau value, it would follow that two-thirds of the lactate has a transit time of 3 min or less, and that one-third has a transit time of more than 3 min. From Table 1 it is seen that about 50% of the dose has been detritiated in the A-VC mode in the first 1 min. The maximal radioactivity in plasma water was attained in about 1 min. It appears that most of the lactate is turned over rapidly, but the turnover is not uniform in all body compartments and a relatively small part has a lower turnover rate.

The mass of lactate is the product of replacement rate and mean transit time. This approach has been rarely used. Conventionally lactate mass has been estimated by multiplying plasma concentration by an arbitrarily assumed volume of distribution. Our findings agree with a mean volume of about 65% of body weight; however, there was considerable variability in lactate space (Table 2). With [^{14}C]lactate the rather prolonged time to approach constant specific radioactivity in infusion, and the rather long mean transit time, about 1 h, were unexpected. It appears that lactate carbon exchanges with a large body of carbon, of the order of 500 mg/kg body wt. This is more than the combined mass of lactate, pyruvate, alanine and glucose. An exchange with some amino acid pools may serve as source of carbon, but further studies are required.

Practical aspects for the study of lactate turnover

The rapid very early decay of ^3H -labelled lactate specific radioactivity causes experimental difficulties with bolus injection. This is most pronounced in the V-A mode. This is also the case with other ^3H -labelled compounds, such as alanine and glycerol. Kallai-Sanfacon *et al.* (1978) recommended the use of a power function to obtain by extrapolation the early part of specific-radioactivity curves. However, this method requires very early sampling to obtain the time of maximal specific radioactivity. Continuous infusion with ^3H -labelled compounds is much easier and more reliable than is single injection. The determination of the kinetics and of the coefficients and exponents for exponential curve-fitting is much easier from wash-out curves.

The attainment of steady state is rather slow with infusion of [^{14}C]lactate. A priming dose equivalent to 150–200 min of infusion is required. The specific radioactivity of glucose should be reasonably constant when isotopic steady state is approached. The wash-out curve for [^{14}C]lactate is most convenient for obtaining the kinetic parameters of this curve, and it permits estimates of the priming dose and time required to approach sufficiently closely to isotopic steady state.

Because of the high rate of carbon cycling, the use of [^{14}C]lactate alone yields little meaningful information on lactate turnover and incorporation into products. However, the use of [U- ^{14}C]glucose and [U- ^{14}C]lactate administered in sequential experiments to the same animal permits an estimate of recycling between these compounds and significant estimates of rates of turnover and interconversion (Depocas & Freitas, 1970). This approach was used by Freminet & Poyart (1975); however, they employed the V-A mode. Kusaka & Ui (1977) employed ^{14}C -labelled glucose, lactate and alanine in such a study, but did not use the A-VC mode. The use of compounds doubly labelled with ^3H and ^{14}C would, with a little extra work, enhance the information obtainable from such studies.

It appears that our study is the first to point out the great significance of sites of tracer injection and sampling in the study of the tracer kinetics. The mode of tracer administration seems to make little difference with compounds of slow turnover, but is of great importance in the study of lactate, alanine, glycerol and probably other compounds with rapid turnover that are present in both intracellular and extracellular space. The possibility to sample a system at two sites should lead to better interpretation of tracer kinetics and more complex knowledge on the metabolism of these compounds. Unfortunately, the use of two modes also doubles the experimental work, and complicates the interpretation. Our findings support the conclusion that,

for employment of a single method of tracer administration, the A-VC mode is, within the limitations of the underlying assumptions of tracer theory, the method of choice to study lactate turnover. Most previous studies of lactate and alanine, and similar compounds, employed the V-A mode, and most of the published results require re-examination. It is to be hoped that future studies will integrate the results of the two modes, and lead to a more realistic model of lactate metabolism than we have now.

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