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The determination of lactate turnover *in vivo* with ^3H- and ^{14}C-labelled lactate

The significance of sites of tracer administration and sampling

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L-[3-^3H,U-^{14}C]Lactate was administered to starved rats either as a bolus or by continuous infusion. Tracer administration was performed two ways: injection into the vena cava and sampling from the aorta (V–A mode), or injection into the aorta and sampling from the vena cava (A–VC mode). The specific-radioactivity curves after infusion or injection differed markedly with the two procedures. However, the specific radioactivities of ^{14}C-labelled glucose derived from [U-^{14}C]lactate were similar in the two modes. The apparent turnover rates of lactate calculated from the ^3H specific-radioactivity curves in the V–A mode were about half those obtained from the ^3H specific-radioactivity curves in the A–VC mode. The apparent contribution of lactate carbon to glucose carbon calculated from specific-radioactivity curves of the A–VC mode was greater than that obtained from the V–A mode. The apparent recycling of lactate carbon calculated from the specific radioactivities for [U-^{14}C]- and [3-^3H]-lactate was greater in the A–VC mode than the V–A mode. [U-^{14}C]Glucose was administered in the two modes, but in contrast with lactate the specific radioactivities were only slightly different. An analysis to account for these observations is presented. It is shown that the two modes represent sampling from different pools of lactate. The significance of sites of tracer administration and sampling for the interpretation of tracer kinetics of compounds present in intracellular and extracellular spaces, and with a high turnover rate, is discussed. We propose that for such compounds, including lactate, alanine and glycerol, the widely used V–A mode leads to a marked underestimate of replacement, mass and carbon recycling, and that the A–VC mode is the preferred method for the assessment of these parameters.

We have previously introduced the use of [^{14}C]- and [^3H]-glucose to measure the true rates of synthesis and replacement and the recycling of glucose carbon atoms (for review see Katz, 1979). We have now applied this approach to the turnover of lactate, employing bolus injection and continuous infusion of lactate simultaneously labelled with ^{14}C and ^3H. In previous studies in our laboratory we have usually administered the labelled compound into the aorta and sampled from the vena cava. The rationale for this procedure was that it assures a uniform supply of tracer to all tissues and that the sample represents the mean specific radioactivity in the venous effluent. In the present study we found it necessary to follow lactate specific radioactivity within seconds after administration of the labelled compound. This is much easier when blood is dripping spontaneously from an artery. Tracer administration into a vein and collection of arterial blood has been used in the great majority of published studies in animals. We observed with this procedure a precipitous decline in specific radioactivity of arterial lactate within seconds of injection. There were difficulties in the interpretation of the results, and we thus investigated the effects of sites of tracer administration and sampling on the kinetics of labelled lactate. We designate here the injection or infusion of tracer into a vein and sampling from the artery as the V–A mode and the administration of tracer into the aorta and sampling from the vena cava as the A–VC mode. We found a marked difference in kinetics of [^3H]- and [^{14}C]-lactate in the two modes. The apparent replacement rates and other parameters of lactate metabolism calculated by conventional procedures differed greatly for the two modes.

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In the present paper we present our observations and provide an explanation for the different kinetics in the two modes. We show that conventional calculations based on curves in the commonly used V–A mode greatly underestimate the parameters of lactate metabolism, and that of other compounds such as alanine and glycerol with rapid fractional turnover. In the present paper our concern is with the effects of the site of tracer administration and of sampling on the assessment of the parameters of lactate metabolism. Quantitative determinations of lactate turnover and mass of lactate carbon recycling and the contribution to glucose carbon from studies with ^{3}H- and ^{14}C-labelled lactate are presented in the following paper (Okajima *et al.*, 1981).

Methods

Experimental design

Male rats of the Sprague–Dawley strain (body wt. 250–300g) were used, and were starved for about 20h before administration of the labelled compound. From 3 to 5 days before the experiment indwelling catheters were implanted (Katz *et al.*, 1974), through the carotid artery and jugular vein into the aorta and vena cava. In some experiments a catheter was also implanted in the femoral artery. It was either cannulated at the same time as the above vessels, or in some experiments the femoral artery was cannulated just before the experiment, and the rats were kept anaesthetized during the experiment.

The dose for single injection per rat was about 15×10^6 c.p.m. for [3-^{3}H]lactate and 5×10^6 c.p.m. for [U-^{14}C]lactate. For continuous unprimed infusion the dose was 15×10^4 c.p.m./min for [3-^{3}H]lactate and 5×10^4 c.p.m./min for [U-^{14}C]lactate. After infusion was terminated, sampling was continued to obtain the washout curves for glucose and lactate. Samples (0.2–0.5ml) of blood were collected. In experiments with single injection sampling was started within seconds after injection. Arterial blood continuously dripped into small preweighed tubes, and samples were collected at intervals from 3 to 20s for up to 2min. Fewer venous samples could be obtained within the first minute after injection.

Labelled lactate

[3-^{3}H]Pyruvate was made by exchange with ^{3}HOH (Rose, 1960). L-[3-^{3}H]Lactate was prepared by the reduction of [3-^{3}H]pyruvate with NADH (Rognstad & Wals, 1976). Sodium pyruvate (50μmol) plus 10μl of 12M-HCl was incubated in 50μl of ^{3}HOH (5Ci/ml) in a stoppered tube for 2h in a boiling-water bath. The [3-^{3}H]pyruvate was used directly, after neutralization, for enzymic reduction to L-[3-^{3}H]lactate. The lactate was purified as described below. L-[U-^{14}C]Lactate was purchased from Amersham–Searle (Chicago, IL, U.S.A.).

Specific radioactivity was determined either in whole blood or in plasma deproteinized with perchloric acid, and the protein-free supernatant was then neutralized with KOH. Samples were taken for glucose and lactate assay. Most of the sample was passed through three small tandem resin columns. The top column contained 0.2ml of Dowex 50 (H^+ form; 100mesh), the middle 0.4ml of Dowex 1 (acetate form; 200mesh) and the bottom column 0.3ml of Dowex 1 (borate form). Washings (10ml) contained the labelled water. The top column was eluted with 5ml of 2M-NH_3 to remove the amino acids. The columns were separated, the middle column was washed with 5ml of 0.25M-acetic acid to remove 3-hydroxybutyric acid, and the lactate was eluted with 4ml of 2M-acetic acid. Then 0.1ml of 0.1M-NaOH was added, and the solution was evaporated to dryness. The bottom column was washed with 5ml of 16mM-NH_4BH_4 to remove any glycerol, the glucose was eluted with 4ml of 0.5M-acetic acid and the eluate was evaporated to dryness.

Methods for the analysis of glucose and lactate and radioactivity assay were as described by Rognstad & Wals (1976). In view of the low radioactivity of the ^{3}H-labelled compounds a reliable measure of background radioactivity and of corrections for cross-over are important. Results are presented as c.p.m./mg of glucose or lactate or as c.p.m./ml. All data are normalized to a dose for single injection of 10000c.p.m./kg body wt. and to the infusion of 1000c.p.m./min per kg body wt.

Results

To compare the kinetics of labelled lactate in the two modes, either rats were injected with [3-^{3}H]lactate into the vena cava and blood was collected from aorta (V–A mode), or animals were injected into the aorta and blood was collected from the vena cava (A–VC mode). In Fig. 1 the specific radioactivities of blood lactate in the two modes are compared. When [3-^{3}H]lactate was injected into the vein the specific radioactivity of arterial lactate increased very rapidly and attained a maximum before 5s. The specific radioactivity at maximum was 20–30% of the dose per mg of lactate. The radioactivity decreased precipitously to about 5% of dose per mg of lactate within the next 5–6s and more slowly to about 0.5% of dose per mg of lactate at 2min after injection. It was about 0.05% of dose per mg of lactate at 10min and became barely detectable at 60min.

The early kinetics of the specific radioactivity in the opposite A–VC mode were radically different. When [3-^{3}H]lactate was injected into the aorta maximal radioactivity in venous blood was 5–6% of the dose per mg of lactate, which was attained 10–15s after injection, and decline was much

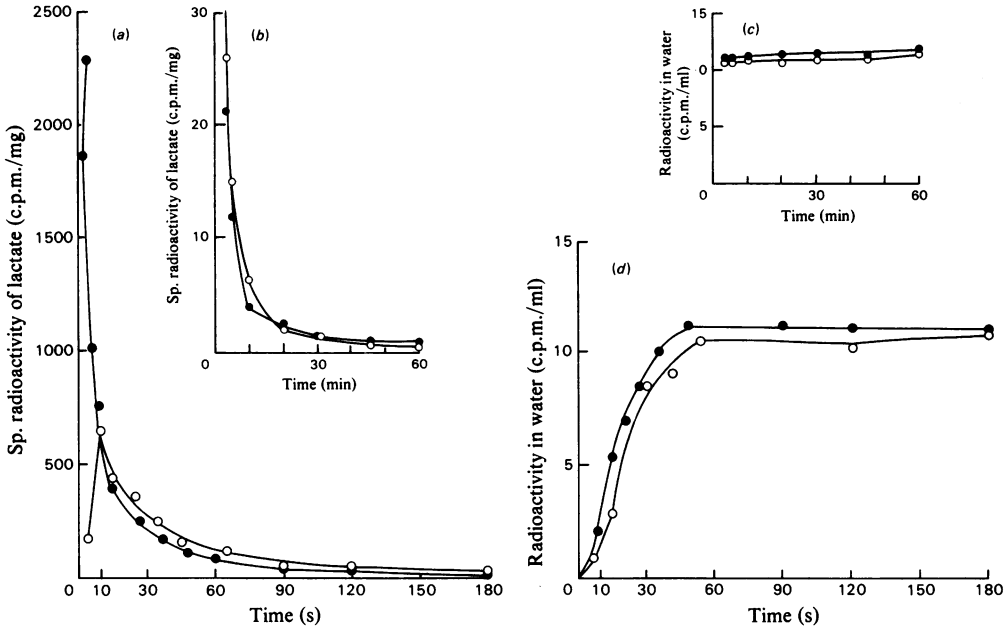

Fig. 1. *Specific radioactivities of blood lactate and radioactivity in plasma water after injection of* [3-^{3}H]*lactate* ●, Injection into the vena cava and blood sampled from the aorta (V–A mode) (average for four rats); ○, injection into aorta and sampling from the vena cava (A–VC mode) (average for three rats). (*a*) and (*b*) Specific radioactivities of blood lactate; (*c*) and (*d*) radioactivity in plasma water. Radioactivities were normalized to an injected dose of 10000 c.p.m./kg body wt.

slower. After about 1 min the specific-radioactivity curves for the two modes became similar. Of special importance are the areas subtended by the specific-radioactivity curves. Conventionally the replacement rate of a compound in steady state is obtained by dividing the injected dose by the area, from zero to infinity, under the specific-radioactivity curve. The area under the curve for arterial blood was twice or more than that under the curve for the venous blood, so that there was a serious discrepancy in the turnover rates obtained by the two modes. A major fraction of the area under the specific-radioactivity curves in venous blood was generated very early after injection. The fraction up to 2 min accounted for 70–80% of the total area subtended by the curve for [3-^{3}H]lactate. With [U-^{14}C]lactate about 40% of the total area was subtended by the curve at 2 min (results not shown).

The detritiation of [3-^{3}H]lactate appeared to be very rapid in both modes (Figs. 1*c* and 1*d*). Near-maximal radioactivity of plasma water was attained at about 1 min after injection and changed little thereafter. Thus the greater part of the injected dose of ^{3}H was released within the first minute as water. The catabolism of [U-^{14}C]lactate was also very rapid. Maximal radioactivity in plasma glucose from [U-^{14}C]lactate was attained 2–4 min after injection (results not shown).

The early decrease in specific radioactivity is so rapid that, if sampling is delayed by a minute or so after injection, the early part of the curves will be missed. This is most pronounced for ^{3}H-labelled lactate in the V–A mode. Extrapolation of the curve will greatly underestimate the intercept on the ordinate and the total area under the curve. Fitting the early part by exponential equations is, even if there is early sampling, subject to great experimental error. Underestimation of the area leads to an overestimate of replacement rate as commonly calculated from the dose/area ratio, and to a large discrepancy in replacement rates as estimated from injection and infusion. Such a discrepancy, by a factor of 2 or more, was noted by Kallai-Sanfacon *et al.* (1978) in their study of glycerol turnover in dogs. [2-^{3}H]Glycerol was administered in the V–A mode by injection and infusion. The decay of the specific radioactivity after injection was precipitous. As with [3-^{3}H]lactate, they found it impossible to describe adequately the early part of the specific-radioactivity curve by extrapolation. When sampling was begun within seconds after injection, a close agreement in replacement rates for glycerol calculated from injection and infusion was obtained.

In continuous unprimed infusion the specific-radioactivity curves are equal, for unit dose, to the integral of the curves with single injection, or in

other words represent a plot of the areas under the specific-radioactivity curves (Shipley & Clark, 1972; Steele, 1971; Katz *et al.*, 1974). Thus the plateau values of specific radioactivity in infusion in the V–A mode would be expected to be much greater than that in the A–V mode. This was indeed observed (Okajima *et al.*, 1981). We wished to compare the two modes in a single animal. In the V–A mode blood is sampled at a site between the site of injection and the capillary bed, whereas in the A–VC mode the sampled blood is the effluent from the tissues. It is thus possible to compare the two modes in one experiment by sampling blood simultaneously from a vein and an artery. Thus [3-^{3}H,U-^{14}C]lactate was administered into the aorta, and blood was taken from the femoral artery and from the vena cava, or alternatively the [3-^{3}H,U-^{14}C]lactate was administered into the femoral artery and blood was taken from the aorta and vena cava. Since early sampling is not essential for obtaining the plateau, it was convenient to compare the two modes by administering the labelled lactate by continuous unprimed infusion.

In Fig. 2 we present one typical experiment with [3-^{3}H,U-^{14}C]lactate infused into the aorta and sampling from femoral artery and vena cava. In most experiments with double sampling there was after a 1–2h period an increase in plasma lactate, but the concentrations in arterial and venous blood remained equal. The increase may be due to the strain imposed by double sampling. Barbital anaesthesia did not abolish the increase. The difference in the kinetics of lactate specific-radioactivity in venous and arterial blood was pronounced whether lactate concentration increased or not.

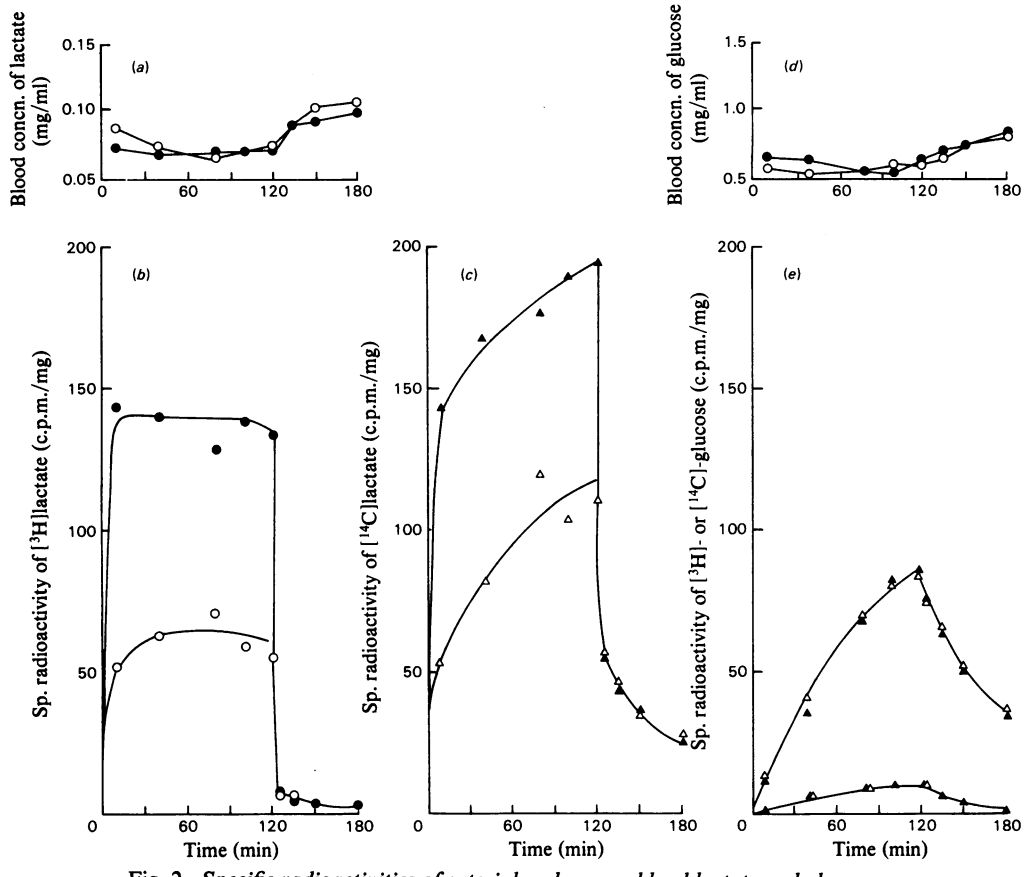

Fig. 2. *Specific radioactivities of arterial and venous blood lactate and glucose*

One rat was infused with [3-^{3}H,U-^{14}C]lactate into the aorta. ● and ▲, Blood sampled from femoral artery; ○ and △, blood sampled from the vena cava. (*a*) Concentration of blood lactate; (*b*) specific radioactivity of ^{3}H-labelled lactate; (*c*) specific radioactivity of ^{14}C-labelled lactate; (*d*) concentration of blood glucose; (*e*) specific radioactivities of ^{14}C-labelled glucose (upper curves) and of ^{3}H-labelled glucose (lower curves). Specific radioactivities were normalized to an infused dose of 1000c.p.m./min per kg body wt.

The specific-radioactivity curves resemble closely the plot of the areas under the specific-radioactivity curves with the two modes by single injection (Fig. 1). The slope of the [^{3}H]lactate curve from the femoral artery increased very steeply, and the plateau was approached, within experimental error (±10%), within a few minutes. The slope of [^{3}H]lactate curve from the vena cava was less steep, and plateau was essentially attained by 30min. The plateau value in arterial blood was in this animal 2.5 times that in venous blood, and values in this range were obtained in a large series of animals. With [U-^{14}C]lactate the specific radioactivities in both modes were still increasing rapidly at the end of a 2h period in both arterial and venous blood, but it is apparent that the plateau value in the femoral artery would greatly exceed that in the vena cava. When infusion was discontinued the specific radioactivities of [^{3}H]- and [^{14}C]-lactate in arterial and venous blood became virtually identical within 1min. Interestingly, in contrast with lactate, the specific radioactivities of glucose derived from either [U-^{14}C]- or [3-^{3}H]-lactate were nearly identical in artery and vein (Fig. 2*e*).

When [3-^{3}H,U-^{14}C]lactate was infused into the femoral artery and blood was sampled from the aorta and vena cava, there was little difference in the specific-radioactivity curves for arterial and venous lactate (Fig. 3). The kinetics were very similar to that of the curves obtained in the venous blood in the V–A mode (Fig. 2).

It was decided to investigate whether the marked difference in the specific radioactivities of arterial and venous lactate obtained in the V–A mode of injection would also hold for glucose. When [6-^{3}H,U-^{14}C]glucose was administered and sampled exactly as in the experiments of Fig. 2, only small differences in the kinetics and plateau values of arterial and venous glucose were obtained (Fig. 4). Thus the kinetics of labelled glucose are quite different from those of lactate.

It is apparent that, according to the mode of administration and sampling, rather different rates for lactate replacement would be calculated. We illustrate this with [3-^{3}H]lactate, where plateaus of specific radioactivity were attained. In this experiment (Fig. 2) the apparent replacement rate of lactate from the plateau value in the arterial blood was 7.1mg/min per kg body wt., as compared with 16mg/min per kg from that in venous blood.

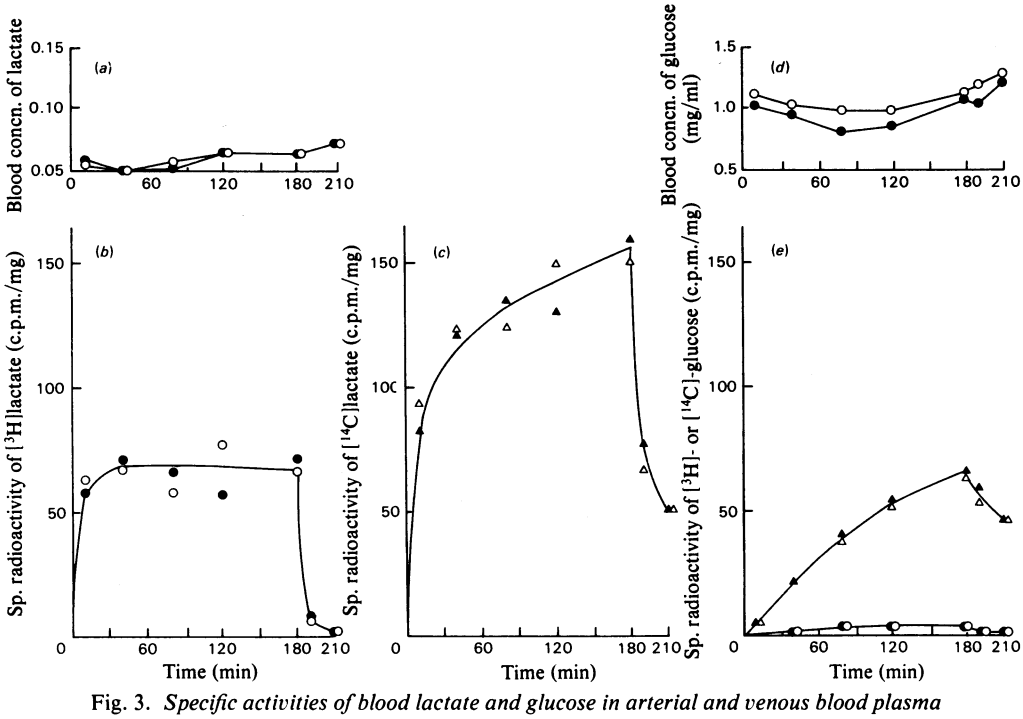

Fig. 3. *Specific activities of blood lactate and glucose in arterial and venous blood plasma*

One rat was infused with [3-^{3}H,U-^{14}C]lactate into the femoral artery. ● and ▲, blood sampled from the aorta; ○ and △, blood sampled from the vena cava. (*a*) Concentration of lactate; (*b*) specific radioactivity of [3-^{3}H]lactate; (*c*) specific radioactivity of [^{14}C]lactate; (*d*) concentration of glucose; (*e*) specific radioactivity of glucose [▲ and △ (upper curves), [^{14}C]glucose; ● and ○ (lower curves), [^{3}H]glucose]. Infused dose was normalized as in Figure 2.

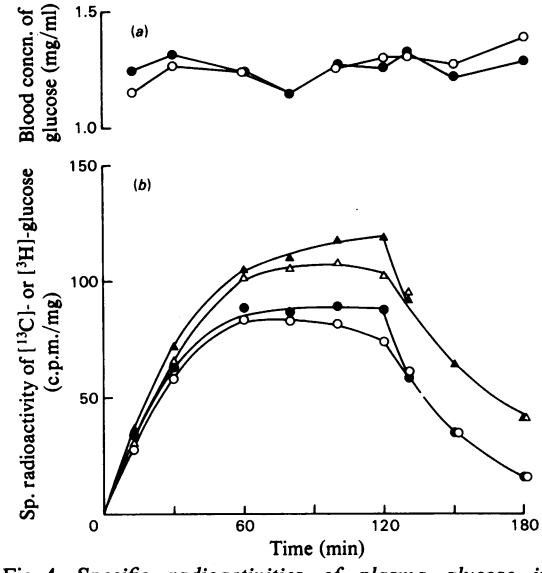

Fig. 4. *Specific radioactivities of plasma glucose in arterial and venous blood*

[6-^{3}H,U-^{14}C]Glucose was infused into the aorta. (*a*) Concentration of blood glucose; (*b*) specific radioactivity of ^{14}C- or ^{3}H-labelled glucose. ● and ▲, Blood sampled from femoral artery; ○ and △, blood sampled from vena cava. ▲ and △, Specific radioactivities of ^{14}C-labelled glucose; ● and ○, specific radioactivities of ^{3}H-labelled glucose. Specific radioactivities were normalized to an infused dose of 1000 c.p.m./min per kg body wt.

On the other hand, in the A–VC mode (Fig. 3) both venous and arterial blood yield a replacement rate of about 15 mg/min per kg body wt.

It is also shown by Fig. 2 that the ratio of specific radioactivity of lactate to that of glucose as determined from the arterial blood is much less than the ratio of the specific radioactivities of lactate and glucose in venous blood. The difference is also marked in the apparent recycling of lactate carbon, as estimated from the ratios of the plateau values of specific radioactivities of [^{14}C]- and [^{3}H]-lactate. In the experiments of Figs. 2 and 3 plateaus for [^{14}C]lactate were not attained, but the marked difference in apparent recycling in the two modes is obvious. In the V–A mode the ^{3}H/^{14}C ratio of plateaus for arterial lactate is about 0.65, but for the venous lactate in the A–VC mode the ^{3}H/^{14}C ratio is about 0.4. The apparent recycling of lactate carbon would thus be in the A–VC mode nearly twice that in the V–A mode.

Discussion

Model to account for the two modes

Our results show that the specific-radioactivity curves for labelled lactate vary according to the mode of isotope administration and sampling, and that the calculated parameters of lactate metabolism would greatly differ in the two experimental modes. To explain our findings it is necessary to consider a model of lactate distribution in the body. Lactate is present in all body fluids and is synthesized and catabolized in numerous tissues. The obvious major physiological divisions of the lactate space are the circulating blood, the extravascular fluid, and the lactate in cells. A schematic model is presented in Fig. 5(*a*). The greater part of body lactate is in the extravascular and intracellular fluid, which is designated as compartment I. This probably is not a 'well-mixed' compartment. This contains also lactate in small blood vessels, the capillary bed and portal blood. The concentration and specific radioactivity of lactate may vary in the different regions, where lactate may be formed and utilized at different rates. (For example lactate concentration may be high in muscles of a working limb, and low in a limb at rest.) Compartment II represents the lactate space in the circulation, including a small amount in extravascular and cellular fluid of heart and lung. This compartment is likely to be a well-mixed pool. In Fig. 5(*b*), the anatomical scheme has been simplified to a compartmental model of two exchanging pools. Lactate synthesis (from carbohydrate, CO_2, lipid etc.) and utilization is essentially confined to pool I, which is depicted with one entry for newly formed lactate and one outflow (loss). There could be some lactate uptake or output in the tissues of compartment II, and lactate is formed by erythrocytes, but the rate is likely to be much smaller as compared with turnover in the major body tissues, and is neglected in the model. The model of Fig. 5(*b*) differs from conventional multicompartmental representation by having separate flow channels in opposite directions between the pools. Inspection of Fig. 5(*a*) shows that injection into a vein from point a_4, and sampling from an artery up to point b_3, constitutes injection and sampling from pool II of Fig. 5(*b*). On the other hand injection into an artery supplying all of pool I (point b_2) and sampling the circulation between b_4 and b_2 constitutes in effect injection into pool I and sampling of effluent from the same pool. This is brought out more clearly by the model in Fig. 5(*b*). When injected into the aorta, lactate of equal specific radioactivity is supplied to all tissues, and that of lactate in the venous effluent taken from the vena cava closely approximates the mean specific radioactivity of lactate in compartment I. Thus the V–A mode of application of labelled lactate represents essentially injection into compartment II, whereas the A–VC mode represents injection into compartment I. Multiple sampling as shown in Fig. 2 (see the Results section) represents injection at point a_2 and withdrawal at points b_3 and b_1, in effect sampling of compartments II and I respectively.

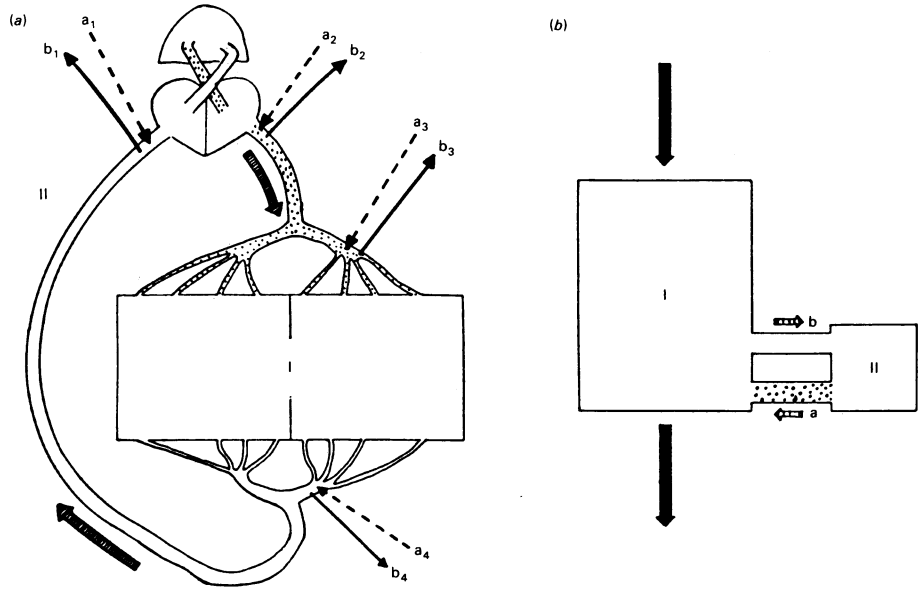

Fig. 5. *Model of the lactate system*

(*a*) Schematic diagram showing lactate in the circulation (II) and lactate in extravascular and cellular water (I). Arterial vessels are stippled. Pool I is divided to indicate the heterogeneity of lactate space. (*b*) Two-compartmental model of lactate metabolism. Compartment I constitutes extravascular and intracellular space. A single entry and exit is assumed. Compartment II constitutes lactate in blood (including extravascular lactate in heart and lung).

Multiple sampling in the experiment of Fig. 3 represents injections at point a_3 and sampling at points b_1 and b_2. Representation of the physiological system by a multicompartmental model with injection and sampling of the same pool is only a rough approximation, but is essential for analysis.

The features of the observed kinetics in the two modes are best explained by a numerical example with a two-pool system, with one inflow and outflow from the same pool (mamillary system). The lactate mass in the side pool (no entry or exit to the outside, corresponding to the vascular compartment of Fig. 5), was taken to be one-quarter of that in pool I. Total mass was 250 and replacement rate 20 per unit time (arbitrary mass units). In Fig. 6 we present the specific-radioactivity curves after injection and infusion into each of these pools for both the injected and the non-injected compartments. [For solution of the differential equations, see Steele (1971).] When injected into pool II, the initial specific radioactivity is very high and decays very rapidly in the early period. The area under this curve (for a dose of 10000c.p.m.) is 750 units. The curve for pool I increases rapidly to a maximum and declines, and after a short period resembles closely the curve for pool II. The area subtended by the curve for pool I is 500. Thus, by using the conventional computation for turnover (dose/area), the curve for pool II yields the value of 13, rather than the correct value of 20 units obtained from the curve for pool I. On the other hand, when injection of the labelled lactate is into pool I (A–V mode), the areas under the curves from both pools are identical and equal 500, both providing a correct value for the replacement rate. It should be noted that, after a short period, the curves for the two pools are quite similar. In practice it is difficult to distinguish between these two curves.

The curves for continuous infusion are also presented for the two modes. When labelled lactate is infused into pool II, the ascending curves for pools II and I are quite different (Fig. 6*a*), and attain respectively plateaus of 75 and 50 (infused dose 1000c.p.m./unit time). However, the wash-out curves for the two pools became very similar a short period after infusion is discontinued. When labelled lactate is injected into pool I the curves from the two pools are similar, and in practice could not be distinguished and approach a plateau of 50, yielding the correct values for the replacement rate.

The replacement rate, R, and total mass, M, of the mamillary system may be calculated by using suitable methods from the curves obtained with tracer administration and sampling of either pool I or pool II. The methods based on the curves from pool I are in wide use. R and M may be obtained either graphically or after fitting the curves with

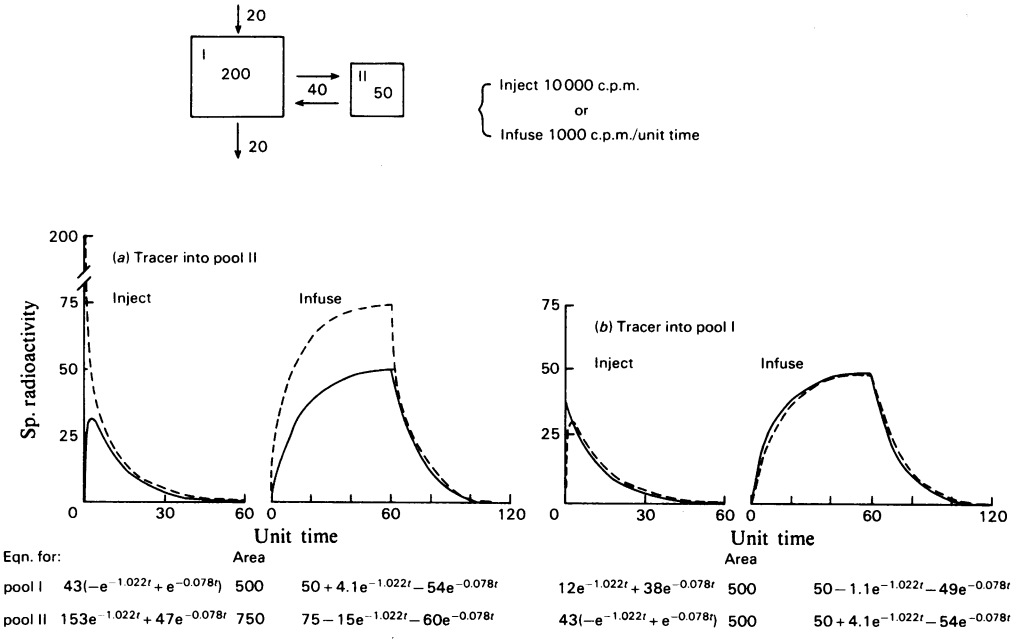

Fig. 6. *Specific-radioactivity curves after bolus injection or continuous infusion into the two compartments of the mamillary system*
For details see the text. ——, Pool I; – – – –, pool II.

exponential expressions. The exponential curve after bolus injection of a dose D is of the form:

$$\text{S.A.} = \sum A_i e^{-\alpha_i t}$$

and that from infusion at a rate J is of the form

$$\text{S.A.} = \sum B_i - B_i e^{-\alpha_i t}$$

where S.A. represents specific radioactivity and

$$B_i = (A_i/\alpha_i) \cdot (J/D).$$

The expressions are (see Katz *et al.*, 1974):

$$R = \frac{D}{(\text{Area})} = \frac{D}{\sum A_i/\alpha_i} \equiv \frac{J}{[\text{Plateau}]} = \frac{J}{\sum B_i} \quad (1)$$

$$M = \frac{D \int_0^\infty \text{S.A.}\,(t)\,dt}{(\text{Area})^2} = \frac{D \sum A_i/\alpha_i^2}{(\sum A_i/\alpha_i)^2}$$

$$\equiv \frac{[\text{Area}]J}{[\text{Plateau}]^2} = \frac{J \sum B_i/\alpha_i}{(\sum B_i)^2} \quad (2)$$

(Area) in round brackets refers to the area under the specific-radioactivity curve after single injection, and [Area] in square brackets refers to the area under the wash-out curve, after infusion is terminated. This area equals that bounded by the curve, the plateau (asymptote) and the ordinate.

It should be noted that, when tracer is administered into pool I, the replacement rate may be obtained by eqn. (1) from the curves from either the injected or the non-injected pool. In general, for valid results in a multiple-pool system, both the tracer and tracee should pass through the sampled pool. When inflow and outflow are restricted to the injected pool, correct values of replacement rate by eqn. (1) are obtained, wherever the site of sampling (see below). Also, in a mamillary model, a correct value for replacement by eqn. (1) is always obtained if sampling is in the non-injected pool (whether tracer is administered into pool I or II), as illustrated in the example. Eqn. (2) is strictly valid only for the curve for pool I, but its use will lead frequently with the curve from pool II only to a moderate overestimate of mass. Graphically mass is most readily computed from the plateau and the area of the wash-out curve after infusion. As seen from Fig. 6, when infusion is into pool I the areas of the wash-out curves differ only slightly, and the use of the curve from pool II (when infusion is into pool I) will yield a mass of 275 units, or a 10% overestimate.

No graphical method has been described to obtain R and M from the curve when tracer administration and sampling is into the side pool of a mamillary system, and the curve has to be expressed by an

exponential expression (pool II of Fig. 6). The equations for replacement and mass are presented below for an exponential expression with two terms for bolus injection, and as general expressions with n exponential terms:

$$R = \frac{D(A_1\alpha_1 = A_2\alpha_2)\ \alpha_1\alpha_2}{A_1A_2(\alpha_1-\alpha_2)^2} = \frac{D\sum A_i\alpha_i}{\sum_{ij}\left(\frac{A_iA_j}{\alpha_i\ \alpha_j}\right)(\alpha_i-\alpha_j)^2} \quad (3)$$

$$M = \frac{D(A_1\alpha_1^2 + A_2\alpha_2^2)}{A_1A_2(\alpha_1-\alpha_2)^2} = \frac{D\sum A_i\alpha_i^2}{\sum_{ij}A_iA_j(\alpha_i-\alpha_j)^2} \quad (4)$$

Corresponding equations for continuous infusion may be obtained by using the transformation of the coefficients shown above.

As an illustration the application of these equations to our example is shown. By inserting the values of the coefficients and exponents for the curve for pool II we obtain the correct values:

$$R = \frac{10000(153\times 1.022 + 47\times 0.078)\times 1.022\times 0.078}{153\times 47(1.022-0.078)^2} = 20$$

$$M = \frac{10000(153\times 1.022^2 + 47\times 0.078^2)}{153.47(1.022-0.078)^2} = 250$$

Use of eqns. (1) and (2) with this curve would lead to a marked underestimate of R (13 units) and M (140 units). On the other hand, use of eqns. (3) and (4) for the curve for pool I will lead to an overestimate of R and M (30 and 314 units respectively).

The model of Fig. 6 is compatible with our observation that the specific-radioactivity curves for glucose are quite similar for both modes. The difference in the areas under the curves or plateau values for pools I or II depends on the ratio of replacement (outflow) to that of the exchange between the pools. In the example of Fig. 6 this was $(40+20)/20 = 1.5$, the same as the ratio of the plateaus (750/500) or of the areas. The fractional rate of glucose utilization is less by one order of magnitude than that of lactate, but the rate of exchange between pools is likely to be much the same for the two compounds. If the outflow in the example of Fig. 6 were 4 rather than 20 units of mass per unit time, the ratio would be $(40+4)/40 = 1.1$ and the difference in the rate of replacement calculated by the two modes would be 10%, similar to the difference seen in Fig. 4.

There is a strong resemblance between the curve pattern of this example and that in our experiments. The experimental curves shown in Figs. 1 and 2 parallel those when injection was into pool II in the model of Fig. 6. When tracer is administered into pool I there is very little difference in the curves from pools I and II. Also, in our experiments (Fig. 2) after lactate administration into a peripheral artery there is virtually no difference in the specific-radioactivity curves from aorta and vena cava. This similarity in pattern is suggestive, but not sufficient proof for the validity of the model, and there may be more satisfactory ones. The model considered here was a mamillary one, with inflow and outflow from the same pool, with the second pool being a side-pocket. A second two-compartment model is the catenary, with inflow and outflow from opposite pools. Eqn. (1) is valid for this system whatever the sampled and injected pool is, but eqn. (2) holds only when sampling is from the non-injected pool. If it were assumed that all the synthesis (inflow) of lactate is into one pool (muscle) and all utilization (outflow) is from the second pool (liver), the catenary system would be plausible. However, in this system the same value for replacement rate should be obtained whichever pool is injected and sampled, or there should be no difference between the two modes. To examine further the plausibility of this model, in a series of experiments [3-^{3}H]lactate was infused into the descending aorta distal to the mesenteric and renal arteries, so that in the first passage the tracer passed only through the capillary bed of muscle. Blood was sampled from the vena cava or aortic arch with the same results. The curves obtained in this type of experiment resembled closely the A–VC mode (see Okajima *et al.*, 1981), and the replacement rates were the same as in the A–VC mode. Thus a catenary model is not tenable.

Tracer administration and sampling

To our knowledge there is no study of the metabolism of labelled lactate in animals (rats and dogs) that has used the A–VC mode. Virtually all investigators have employed the V–A mode. For example, in the studies by Depocas *et al.* (1969), Minaire *et al.* (1971), Wiener & Spitzer (1974),

Freminet & Payart (1975), Wolfe *et al.* (1977) and Issekutz *et al.* (1976) [^{14}C]lactate was administered into a vein and blood was taken from an artery. Forbath & Hetenyi (1970) injected into the jugular vein and collected from the vena cava, again a V–A mode. Kusaka & Ui (1977) injected into a femoral vein and collected mixed venous–arterial blood from the tail. The V–A mode has also been predominantly used in animals with alanine and glycerol. On the other hand, in humans the common but not universal practice is tracer injection into an arm vein and drawing blood from the vein on the opposite arm [for example see Searle & Cavalieri (1972) and Kreisberg *et al.* (1970)], which resembles the A–VC mode.

The difference between the two modes applies also to alanine. We found after the injection of [3-^{3}H,U-^{14}C]alanine a pattern for the two modes resembling that of lactate, with the replacement rate in the A–VC mode about twice that in the V–A mode (J. Katz, F. Okajima, M. Chenoweth & A. Dunn, unpublished work). Chiasson *et al.* (1977) with dogs mentions (without giving data and without comment) a large difference in the specific radioactivities of ^{14}C-labelled alanine in arterial and venous blood when tracer was administered into a peripheral vein. The results obtained by Kallai-Sanfacon *et al.* (1978) in dogs leave little doubt that marked difference for the two modes would be found with glycerol. It is most probable that this will also apply to other compounds with a high turnover rate, that are present in both cells and extracellular fluid. It appears that most published values on the turnover of such compounds requires re-examination.

In the following paper (Okajima *et al.*, 1981) we adduce the evidence that calculations based on the A–VC mode provide a much more realistic measure of the parameters of lactate metabolism than does the V–A mode, and previous studies based on that mode have lead to an underestimate of the replacement rate.

The ideal A–VC mode is to supply tracer uniformly to all tissues, and to sample the mixed effluent from all parts of the body. Injection into the aorta and sampling from the vena cava appears to be the closest practical approach to this goal. However, alternative methods, provided that the capillary bed is between the site of tracer administration and the site of sampling, may be adequate. In bolus injection we have used injection and sampling from the aorta (Okajima *et al.*, 1981). With proper precaution there is no contamination of the samples by the injected dose. Only one cannula is required, and obtaining blood from the aorta is easier than from the vein. Also, when tracer was administered into the descending aorta and sampling was from the aortic arch (A–A mode), we found much the same results as in the A–VC mode (Okajima *et al.*, 1981). It is likely that tracer administration into a peripheral artery and sampling from an artery on the opposite limb will yield much the same curves as was used in the present work. When deep cannulation is not practical (as may be the case with humans), this is probably the method of choice. Tracer injection into a peripheral vein and collection from the opposite limb may not be adequate, since the specific radioactivity in the effluent of various body regions may differ. McGuire *et al.* (1976) injected [^{14}C]glucose into the antecubital vein in humans and sampled blood from the artery from that vein on the opposite arm, and from a vein in a heated hand. They usually found little difference in the specific radioactivity of glucose in arterial and venous blood. There was a small but significant difference in the specific radioactivity of the two venous sites. Probably even for the study of glucose turnover the use of the A–VC mode is to be preferred.

The importance of the sites of tracer injection and sampling has been well understood in the study of haemodynamics, such as in the determination of blood flow and blood volume in organs (see, e.g., the monograph by Lassen & Perl, 1979), but has been so far overlooked for the study of tracer kinetics. Blood has been taken as a well-mixed pool, where the locus of injection and withdrawal is immaterial. This concept may hold reasonably well for compounds present in extracellular water (glucose, plasma albumin, drugs etc.) with slow fractional turnover, but, as shown in the present work, cannot be applied to compounds that are largely intracellular and where turnover is high. In such systems the nature of the circulation and the direction of blood flow must be taken into account in experimental design and in constructing realistic models.

The concept of blood as a well-mixed pool has led numerous investigators to disregard the early rapid decline in specific radioactivity after injection as being due to 'mixing', and attribute the mono-exponential decline to 'catabolism'. Thus sampling has frequently been delayed for several minutes after injection. This may lead to serious discrepancies between the replacement rates as obtained from single injection and those from continuous infusion. This is well illustrated by our findings as well as by the results obtained by Kallai-Sanfacon *et al.* (1978) with glycerol. The calculation of a rate of replacement depends on the total area under the specific-radioactivity curve, and it is irrelevant, in theory as in practice, whether the decline is by mixing or by catabolism. Moreover, with lactate a major part of the label is removed very early (see Fig. 1), and at about 2 min after 90% of the labelled lactate has been detritiated or converted into other carbon compounds. It is thus difficult to distinguish the

contributions of redistribution and of catabolism to the decline in specific radioactivity.

Model of lactate metabolism

If lactate kinetics were to conform to a simple mamillary model as in Fig. 5, a calculation of replacement rate and mass from the V–A mode by eqns. (3) and (4) should yield much the same values as calculated from eqns. (1) and (2) from the A–VC mode. In the following paper (Okajima *et al.*, 1981) we present the exponents and coefficients for the two curves. Eqns. (3) and (4) applied to the V–A mode yield much higher values than those from eqns. (1) and (2) for the A–VC mode, and thus the model shown in Fig. 5 does not offer an adequate quantitative description of the lactate system. Steele (1971) has thoroughly examined the applicability of eqns. (1) and (2) to a general two-compartmental mode. With all combinations of inflow and outflow there are nine possible systems where injection and sampling is from the same pool. Eqn. (1) is valid for three out of four configurations with a single inflow and outflow. It also holds when there is inflow and outflow from the sampled pool and either one inflow or one outflow from the non-injected pool. Altogether, eqn. (1) holds for five out of nine possible configurations of a two-compartmental system. Thus, if there were either an inflow or outflow from pool 2 of the model shown in Fig. 5 (but not both), eqn. (1) for the A–VC mode would provide a valid estimate for the replacement rate. However, the validity of eqn. (2) for mass is greatly restricted. It is valid only for pool 1 of a mamillary system, or in only one case out of nine. Steele (1971) has also shown that, for any curve, eqns. (1) and (2) yield the minimal values and eqns. (3) and (4) maximal values for replacement and mass consistent with the experimental curve.

Physiological and kinetic considerations leave little doubt that in the A–VC mode tracer is administered and the effluent sampled from a complex compartment representing the greater part of body lactate, and which contains the major sites of lactate synthesis and catabolism. It is quite unlikely that this can be depicted as a well-mixed pool. There is little doubt that the V–A mode represents injection and sampling of a side pool, and eqns. (1) and (2) are not likely to be valid for this mode.

The A–VC and V–A modes represent sampling of a different site of one system, and this in theory should permit a better definition of the model for lactate metabolism than is possible from sampling a single site. Ideally a model should account quantitatively for both sets of parameters, and yield consistent values from specific-radioactivity curves obtained in the two modes. We have not been able to define a simple multicompartmental model with well-mixed pools that would yield consistent and reasonable values for replacement and mass from both modes. In the following paper we offer (Okajima *et al.*, 1981) some possible reasons for the discrepancy. It is thus uncertain to what extent our calculations, using eqns. (1) and (2) and the A–VC mode, depart from the 'true' values of replacement and mass. However, our results support the conclusion that calculations based on the A–VC mode are much closer to the 'true' values than those based on the frequently employed V–A mode.

Note added in proof (received 27 October 1980)

A report by Reilly & Chandrasena (1977) came lately to our attention. The infused L-[U-^{14}C]lactate intravenously and sampled blood from the jugular vein and carotid artery. They observed that the plateau specific radioactivity in arterial blood was about 25% higher than that in venous blood. They, however, chose the radioactivity in arterial blood (V–A mode) to calculate the rate of lactate turnover.

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