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The Effect of Reduction of Perfusion Rate on Lactate and Oxygen Uptake, Glucose Output and Energy Supply in the Isolated Perfused Liver of Starved Rats

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1. Lactate and O_2 uptake and glucose output were studied in isolated livers from starved rats at perfusate flow rates varying from 100 to 7% of 'normal' (11.25–0.75ml/min per 100g body wt.). 2. With moderate diminution of flow rate, lactate and oxygen uptake fell more slowly than would be expected if uptake purely depended on substrate supply. 3. Use of a mathematical model suggests that the intrinsic capacity of the liver for lactate uptake is unaffected until the flow rate falls below 25% of 'normal'. 4. Some lactate uptake was always observed even at 7% of the 'normal' flow rate. 5. At flow rates below 33% of the 'normal', lactate was increasingly metabolized by pathways other than gluconeogenesis, which became a progressively less important consumer of available O_2. 6. ATP content decreased with diminution of flow rate, but substantially less markedly than did lactate uptake and glucose output. 7. Intracellular pH fell from a mean value of 7.25 at 'normal' flow rate to 7.03 at 7% of the 'normal' flow rate.

In spite of the large number of studies made on the mechanisms of hepatic lactate uptake and gluconeogenesis, little detailed attention has been paid to the specific effect of perfusion rate on these processes, apart from the work of Tashkin *et al.* (1972) in anaesthetized dogs. The subject is of obvious physiological and clinical significance because major falls in hepatic blood flow occur during exercise and shock, situations in which there is a heavy burden of lactate and H^+ ions for removal. *In vivo*, it is likely that, in addition to hepatic blood-flow changes, the hormonal environment also plays a substantial role in determining hepatic lactate metabolism in these circumstances. The use of the isolated perfused liver affords an opportunity of determining the consequences of flow changes independently of acute hormonal effects. In the studies described in the present paper we characterize the effects of graded diminution in flow rate on lactate and O_2 uptake, on glucose output, on adenine nucleotides and on intracellular pH in isolated perfused livers from starved rats. A mathematical model of the events taking place was developed that has proved helpful in the interpretation of results.

Materials and Methods

Animals

Inbred male Glaxo–Wistar rats (body wt. 150–200g) were starved for 48h before the experiments.

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Chemicals

All enzymes, NAD^+, NADH, ATP and ADP were obtained from the Boehringer Corp., Lewes, East Sussex, U.K.; bovine serum albumin (fraction V) and L(+)-lactic acid were from Sigma Chemical Co., Poole, Dorset, U.K.; hydroxy[^{14}C]methylinulin (sp. radioactivity 12.6mCi/mmol) and ^3H_2O (sp. radioactivity approx. 100μCi/ml) were from The Radiochemical Centre, Amersham, Bucks, U.K.; 5,5′-dimethyl[2-^{14}C]oxazolidine-2,4-dione (sp. radioactivity 11mCi/mmol) was from New England Nuclear Corp., Dreieichenhain, West Germany; unlabelled 5,5′-dimethyloxazolidine-2,4-dione was from Baxter Laboratories, Morton Grove, IL, U.S.A. All other chemicals were of the purest grade available commercially.

Methods

Isolated perfused liver preparations were set up as described previously (Cohen *et al.*, 1973), modified to overcome loss of O_2 through the Portex tubing, which was substantial at low flow rates. This was done by establishing a by-pass around the liver arising just proximal to the portal-venous cannula and adjustable by a screw clamp to give the required flow through the liver. Thus high flow proximal to the cannula was maintained even at low liver perfusion rate. The perfusion medium was prepared as described by Iles *et al.* (1977). Expired human erythrocytes (4–5 weeks after donation) were used to give a final packed-cell fraction of approx. 0.17. The pH of the perfusate was maintained at 7.35–7.45

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and pCO_2 at approx. 4.9kPa (Table 1); the pO_2 of the inflow via the portal vein was approx. 60kPa. Sodium L(+)-lactate [prepared by neutralizing L(+)-lactic acid] was added to give an initial concentration of approx. 2.5mM. At the beginning of each experiment 5,5'-dimethyl[2-^{14}C]oxazolidine-2,4-dione, hydroxy[^{14}C]methylinulin and ^3H_2O were added for measurement of intracellular pH (pH_i). The amounts added, together with the quantities of unlabelled 5,5'-dimethyloxazolidine-2,4-dione and inulin, have been previously described (Cohen *et al.*, 1971).

During the first 25min of perfusion the medium was recirculated at a flow rate of 7.5ml/min per 100g body wt., a value that we and other authors (Exton & Park, 1967) have used in previous work with this type of preparation. The flow rate was then either increased to 11.25ml/min per 100g body wt., allowed to remain unchanged or decreased to 3.77, 1.5 or 0.75ml/min per 100g body wt. We have regarded 11.25ml/min per 100g body wt. as nominally 100% of normal flow rate on the basis of hepatic blood-flow measurements made in conscious rats (Yudkin *et al.*, 1976) and allowing for the low packed-cell volume of the perfusate. Precise definition of 'normal flow' is difficult. Apart from the need to allow for the low packed-cell volume, dissolved O_2 at pO_2 of 60kPa adds approx. 15% to the available O_2. However, this is likely to be largely offset by left shift of the O_2-dissociation curve in the expired blood used. The other values of flow rate correspond to 67, 33, 13 and 7% of the 'normal' flow rate. Perfusion was then continued in a non-recirculatory mode for a further 20min. At this point portal and hepatic-venous samples were taken for measurement of pH, pCO_2, pO_2, lactate and glucose concentrations, O_2 content and concentration of radioactive labels; flow was measured by running the venous effluent into a measuring cylinder. The liver was then rapidly excised and clamped between tongs precooled in liquid N_2, ground under liquid N_2 and the frozen powder deproteinized in 0.6M-$HClO_4$. At the highest flow rates the non-recirculating perfusion time was often slightly shortened because of exhaustion of the reservoir, but we have shown that at both 100 and 7% of the 'normal' flow rate the liver is in a steady state between 10 and 20min of non-recirculation, as judged by repeated measurements of lactate uptake and glucose output.

Chemical methods

The methods for determining the concentrations of perfusate lactate and glucose, pH, pCO_2 and pO_2, tissue lactate and radioactivity counting have been previously described (Cohen *et al.*, 1971; Iles *et al.*, 1977). Perfusate O_2 content was measured with a Lex-O_2-Con analyser (Lexington Instruments Corp., Waltham, MA, U.S.A.). Tissue adenine nucleotides were measured by the methods of Jaworek *et al.* (1974).

Calculations

The rates of lactate and O_2 uptake and glucose output were calculated from the portal–hepatic-venous concentration differences and the flow rate. pH_i was calculated as previously described (Cohen *et al.*, 1971) by using hepatic-venous pH as the estimate of extracellular pH. Tissue concentrations of lactate were corrected for admixture with extracellular fluid by using the extracellular-space fraction determined from the measurements of hydroxy[^{14}C]methylinulin and ^3H_2O radioactivities; in these experiments mean cell water was 66% of total tissue water. It was assumed that the concentration of lactate in the extracellular space was equal to that in the venous perfusate, and that ATP, ADP and AMP were entirely intracellular. Results are expressed as means ± S.E.M. Standard two-tailed *t* tests were used for comparison between groups.

Results

Effects of diminution of flow rate on lactate and O_2 uptake and on gluconeogenesis from lactate

Table 1 shows that a decrease in flow rate to 67% of nominal normal flow produced small decrements in lactate uptake and glucose output; the fall was, however, significant only in the case of O_2 uptake. Larger decrease in flow produced more significant falls for all three variables. Fig. 1 shows the results in terms of percentage falls from 'normal' at different percentage flow decrements, and also indicates the expected relationship if lactate and O_2 uptakes were purely dependent on the supply of these substrates to the liver. Clearly lactate and O_2 uptakes fall more slowly than expected on this hypothesis at flow rates between 100% and 33–50% of normal, but at lower flow rates the fall is more rapid than expected. Even at 7% of 'normal' flow rate, lactate and O_2 uptakes are still occurring, albeit much diminished. The last line of Table 1 shows that doubling of the portal-venous lactate concentration at 13% of the normal flow rate increases lactate uptake, but not glucose output or O_2 uptake. Table 1 also shows the mean ratios of lactate uptake/glucose output and O_2 uptake/glucose output at the different flow rates.

Effects of diminution of flow rate on perfusate pH and pCO_2 and on pH_i (Table 2)

Portal-venous pH and pCO_2 were between 7.39 and 7.41 and between 4.7 and 5.2kPa respectively at all flow rates. Portal-venous pO_2 was only occasionally checked, being approx. 60kPa.

Table 1. *Effects of perfusion rate on lactate and O_2 uptake, on glucose output and on intracellular lactate concentration*

Conditions are as given in the Materials and Methods section. Results are means ± S.E.M.; numbers of observations are indicated in parentheses. Significances, indicated by asterisks, are those of differences from the mean under 'normal' flow conditions; *$P<0.05$; **$P<0.01$; ***$P<0.001$. The last line of the Table refers to observations at a flow rate 13% of the normal when the perfusate lactate concentration was approximately doubled; the significances of differences from results at 13% of normal flow rate at the standard lactate concentration of 2mM given in the fourth line of the Table are indicated by † ($P<0.05$), †† ($P<0.01$) and ††† ($P<0.001$).

Flow rate (% of 'normal')	Portal-venous lactate concn. (μmol/g)	Hepatic-venous lactate concn. (μmol/g)	Intracellular lactate concn. (μmol/ml of cell water)	Lactate uptake (μmol/min per 100g body wt.)	Glucose output (μmol/min per 100g body wt.)	O_2 uptake (μmol/min per 100g body wt.)	Lactate uptake / glucose output	O_2 uptake / glucose output
100	2.1 ±0.2 (8)	1.5 ±0.2 (8)	0.86 ±0.06 (5)	7.6 ±0.5 (8)	3.5 ±0.2 (8)	14.3 ±0.6 (6)	2.2 ±0.15 (8)	4.13 ±0.48 (6)
67	1.9 ±0.1 (15)	1.1 ±0.1 (15)	0.64* ±0.05 (13)	6.6 ±0.3 (15)	3.0 ±0.2 (15)	11.8* ±0.5 (7)	2.26 ±0.14 (15)	3.92 ±0.27 (7)
33	2.0 ±0.1 (10)	0.87* ±0.09 (10)	0.65* ±0.07 (8)	4.6*** ±0.4 (10)	2.3** ±0.2 (10)	9.3*** ± 0.4 (8)	2.03 ±0.10 (9)	4.38 ±0.33 (7)
13	2.0 ±0.2 (11)	0.94* ±0.08 (11)	1.01 ±0.14 (7)	1.71*** ±0.2 (11)	1.2*** ±0.1 (11)	5.5*** ±0.3 (11)	1.46** ±0.12 (11)	4.98 ±0.34 (11)
7	2.0 ±0.2 (11)	1.3 ±0.2 (11)	0.98 ±0.08 (7)	0.46*** ±0.06 (10)	0.41*** ±0.05 (10)	2.7*** ±0.2 (6)	1.32** ±0.22 (10)	6.50* ±0.72 (6)
13A	4.1††† ±0.2 (8)	2.6††† ±0.2 (8)	1.26 ±0.20 (3)	2.4† ±0.2 (8)	1.2 ±0.1 (8)	5.7 ±0.6 (4)	2.09†† ±0.13 (8)	5.50 ±0.52 (4)

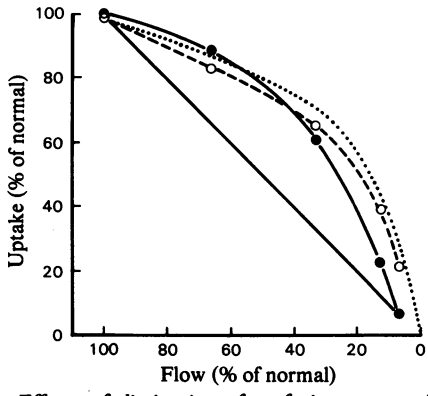

Fig. 1. *Effects of diminution of perfusion rate on lactate uptake* (●) *and* O_2 *uptake* (○)
The conditions are described in the Materials and Methods section. The straight line represents the theoretical relation that would obtain if, at fixed portal-venous lactate or O_2 content, uptake were purely dependent on substrate supply. The dotted line (····) is the theoretical plot of the relationship between flow rate and lactate uptake based on the equation $U = RL_p(1 - e^{-K/R})$ as derived in the Appendix. This model ignores any changes in intrinsic lactate uptake capacity of hepatocytes that may arise from alterations in flow rate [factor (*b*); see the text]. The value of K used was derived from the slope of the straight line drawn through the first three points of Fig. 2.

There is the expected fall in hepatic-venous pO_2 as flow rate decreases; no change in hepatic-venous pH or pCO_2 occurs until flow rates of 7% of the normal are reached, when there is a modest fall in pH and rise in pCO_2. Intracellular pH (pH_i) at normal flow rates was comparable with that found by Lloyd *et al.* (1973) under similar conditions, and was significantly decreased at flow rates of 13% and less of the normal. Table 1 gives values for perfusate and intracellular lactate concentrations; intracellular lactate concentration fell slightly at moderate diminutions of flow rate, but rose to normal values at lower flow rates. To examine the mode of entry of lactate into the liver cell, the regression of the ratio of intracellular to extracellular lactate concentration (L_i/L_e) on the inverse ratio for H^+ activity (H_e/H_i) was examined (Iles *et al.*, 1978); no significant regression was found at any flow rate.

Effects of diminution of flow rate on hepatic concentrations of adenine nucleotides

Table 3 shows that ATP concentration was relatively resistant to ischaemia (in comparison with lactate uptake and glucose output) down to a flow rate of 33% of the normal. Even at a flow rate 13% of the normal, the ATP concentration was still almost 60% of the normal value; a further fall to 23% of the normal value occurred at a flow rate 7% of the normal. Changes in ADP concentration were

Table 2. *Portal- and hepatic-venous pH,* pCO_2 *and* pO_2, O_2 *extraction and uptake and intracellular pH in livers perfused at different flow rates*
Conditions are as given in the Materials and Methods section. For explanation of significances see the legend to Table 1. The last line of the Table refers to observations at a flow rate 13% of the normal when the perfusate lactate concentration was approximately doubled. 'PV' and 'HV' refer to the portal- and hepatic-venous concentrations respectively.

Flow rate (% of normal)	pH		Intracellular pH	pCO_2(kPa)		pO_2 (kPa)	O_2 uptake (μmol/min per 100g body wt.)	O_2 extraction (%)
	PV	HV		PV	HV	HV		
100	7.41 ±0.01 (8)	7.42 ±0.01 (8)	7.25 ±0.02 (5)	5.2 ±0.1 (8)	5.2 ±0.1 (8)	4.5 ±0.1 (4)	14.3 ±0.6 (6)	37.1 ±0.4 (6)
67	7.41 ±0.01 (17)	7.41 ±0.01 (17)	7.27 ±0.04 (8)	4.8 ±0.2 (17)	5.1 ±0.2 (17)	3.1** ±0.2 (16)	11.8* ±0.5 (7)	47.4* ±2.6 (7)
33	7.41 ±0.01 (8)	7.40 ±0.01 (8)	7.22 ±0.06 (7)	4.7 ±0.2 (9)	4.9 ±0.2 (19)	2.7*** ±0.1 (9)	9.3*** ±0.4 (8)	69.4*** ±3.1 (8)
13	7.41 ±0.01 (11)	7.41 ±0.01 (11)	7.13* ±0.04 (9)	4.7 ±0.2 (11)	4.9 ±0.2 (10)	1.7*** ±0.1 (5)	5.5*** ± 0.3 (11)	86.3*** ±1.4 (11)
7	7.39 ±0.02 (10)	7.31** ±0.02 (10)	7.03** ±0.08 (5)	5.1 ±0.2 (10)	6.0* ±0.4 (11)	1.5*** ±0.1 (7)	2.7*** ±0.2 (6)	88.1*** ±3.3 (6)
13A	7.40 ±0.03 (8)	7.38 ±0.03 (8)	7.11 ±0.08 (6)	4.7 ±0.3 (8)	5.1 ±0.2 (8)	1.6 ±0.1 (6)	5.7 ±0.6 (4)	94.6† ±1.0 (4)

Table 3. *Effect of perfusion rate on hepatic concentrations of adenine nucleotides*
Conditions are as given in the Materials and Methods section. For explanation see the legend to Table 1. The last line refers to observations at a flow rate 13% of the normal when the perfusate lactate concentration was approximately doubled.

Flow rate (% of normal)	Hepatic concentration (μmol/ml of cell water)			
	ATP	ADP	AMP	Total adenine nucleotides
100	5.12±0.11 (5)	2.05±0.04 (5)	0.61±0.02 (5)	7.78±0.11 (5)
67	4.67±0.31 (13)	2.00±0.12 (12)	0.56±0.04 (12)	7.62±0.33 (12)
33	4.02±0.22** (8)	2.19±0.16 (7)	0.76±0.07 (7)	6.76±0.21** (7)
13	2.96±0.21*** (7)	2.47±0.13* (6)	1.26±0.08*** (6)	6.81±0.22** (6)
7	1.16±0.08*** (7)	1.94±0.07 (7)	2.01±0.22*** (7)	5.00±0.15*** (7)
13A	3.29±0.25 (7)	2.36±0.13 (3)	1.23±0.16 (3)	7.18±0.21 (3)

unremarkable. AMP concentration showed no significant change until the flow rate was lowered to 13% of the normal, when a sharp rise occurred, and at a flow rate 7% of the normal the rise was 320%. Total adenine nucleotide concentration had decreased to 64% of the normal value at a flow rate 7% of the normal.

Discussion

Fig. 1 demonstrates that, as perfusion rate is lowered, lactate uptake initially declines more slowly than would be expected on the hypothesis that uptake depends purely on lactate supply to the liver. At least two further factors need to be taken into account to explain the shape of the curve for lactate uptake in Fig. 1. The first factor (*a*) is that, although less lactate is supplied to the liver at low flow rates, the lactate that is available remains within the hepatic sinusoids for a longer period than it would at normal flow rate, and there is therefore greater opportunity for uptake. The second factor (*b*) is that presumably when the blood supply is sufficiently diminished there is a decrease in the intrinsic capacity of the cells to take up lactate for gluconeogenesis or oxidation, either due to hypoxia directly or related to failure of wash-out of metabolite(s) that in some way inhibit lactate uptake. In order to deduce from the results to what extent factor (*a*) is determining the shape of the lactate uptake curve and at what diminution of flow rate intrinsic effects on the liver begin to be manifest, we have constructed a simple mathematical model (see the Appendix), which takes into account factor (*a*), but ignores factor (*b*). The main assumption is that along the sinusoid all cells take up lactate at a rate proportional to the concentration of lactate in the portion of the sinusoid immediately adjacent to them. This assumption is justified by the linear relationship between perfusate lactate concentration and lactate uptake shown to exist by Iles (1974, p. 145) at perfusate lactate concentrations in the range 0.5–4mM at 'normal' flow rates. In the Appendix it is shown that the conclusions that we draw below would be unaffected if the proportionality constant varies along the length of the sinusoid for any given set of conditions. This is not unlikely, since Novikoff (1959) found higher activity of lactate dehydrogenase in the centrilobular compared with the periportal region, and Guder & Schmidt (1976) produced evidence that gluconeogenesis was preferentially localized in periportal hepatocytes.

The following relationships may be derived from the model:

$$U = RL_p(1-e^{-K/R}) \quad (1)$$

$$\ln(L_p/L_v) = K/R \quad (2)$$

where U is lactate uptake, R is the flow rate, L_p and L_v are portal- and hepatic-venous lactate concentrations respectively and K is a constant dependent on both the anatomical dimensions of the sinusoid and on the intrinsic capacity of the cells to take up lactate. Since we have no independent measure of K, an absolute test of the model cannot be made, but the general shape of the curve of uptake against flow rate given by eqn. (1) is shown in Fig. 1. This shape is remarkably similar to that described by the experimental points in Fig. 1, even though the model does not take the intrinsic factor (*b*) into account. It is therefore not possible, by using eqn. (1), to assess at what flow rate intrinsic effects become important.

However, eqn. (2) turns out to be more useful from this point of view. This relationship demonstrates that a semi-logarithmic plot of L_p/L_v against $1/R$

should give a straight line through the origin of slope K. Fig. 2 shows a plot of the experimental values of L_p/L_v against $1/R$. Between the values of $1/R$ corresponding to 100 and 25% of the normal flow rate the points lie on a straight line of positive slope, which on extrapolation passes close to the origin, i.e. the expectations of the model are qualitatively obeyed. However, at values of $1/R$ corresponding to flow rates less than 25% of the normal the curve through the experimental points deviated markedly from the straight line; the curve passes through a maximum and then declines towards the abscissa. The most likely explanation of this finding is that intrinsic effects become apparent at flow rates below 25% of the normal and that K is thereby progressively decreased. Although again the theoretical straight line in Fig. 2 is arbitrarily placed because of lack of an independent measure of K, the gross departure from linearity seen at flow rates less than 25% of the normal make it clear that any model of the type considered here breaks down in this region. The model does not attempt to distinguish between intrinsic effects due on the one hand to hypoxia and on the other to failure of wash-out of metabolites at low flow rates; although intracellular pyruvate was not measured in the present work, comparison of the relationship between the intracellular lactate/pyruvate concentration ratio (corrected for changes in pH_i) and flow rate with the experimental curve in Fig. 2 could help to separate the two effects.

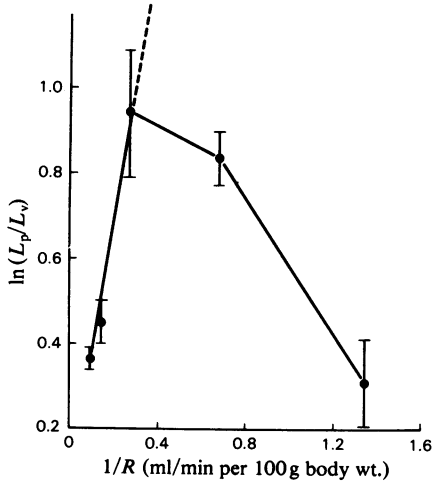

Fig. 2. *Relationship between the reciprocal of the flow rate (R) and $\ln(L_p/L_v)$*

The plot should be a straight line of positive slope through the origin if the assumptions of the model (see the Appendix) were obeyed. The interrupted line is an extrapolation of a straight line drawn through the first three points. The experimental points rapidly fall away from this line at values of $1/R > 0.4$.

The present results may be compared with those obtained by Tashkin *et al.* (1972) in anaesthetized dogs *in vivo*, in which blood flow to the liver was progressively restricted. These workers found no change in lactate uptake until the flow rate was diminished to 25% of the normal, in contrast with the slow but progressive decline at smaller diminutions of flow rate observed in the present work. Tashkin *et al.* (1972) found that lactate uptake began to decline when the limitation of flow had caused hepatic-venous pO_2 to fall below 3.2kPa. In the present studies the first flow rate at which a significant fall in lactate uptake was observed was 33% of the normal, at which the mean hepatic-venous pO_2 was 3.1kPa; in this respect therefore the studies are closely similar. It is also of note that in neither study was lactate output observed even at the lowest flow rates, in contrast with previous findings in ischaemic perfused dog liver, where lactate output occurred at a flow rate of 10% of the control, at which O_2 uptake was 30% of normal (Iles, 1974, p. 55).

The lack of dependence of the lactate-distribution ratio (L_i/L_e) across the cell membrane on the H^+-distribution ratio (H_e/H_i) suggests that at all flow rates the lactate ion is a major form in which lactate enters hepatocytes. However, a component of non-ionic diffusion cannot be excluded, and we have previously presented evidence of its occurrence at extracellular lactate concentrations less than 1.5mM (Iles *et al.*, 1978).

There was a progressive fall in intracellular pH with decreasing flow rate. The fall might well be accounted for by the decrease in lactate uptake and metabolism, since, if lactate entry into hepatocytes were partially in the ionized form (Cohen *et al.*, 1971; Cohen & Iles, 1975), H^+ consumption during subsequent metabolism to glucose or CO_2 and water would be diminished. Breakdown of ATP might also play a part in the lowering of cell pH. The small fall in hepatic-venous pH and rise in venous pCO_2 seen at the lower flow rates could account for only a minor portion of the fall in cell pH (Lloyd *et al.*, 1973; Baron *et al.*, 1978). It should be noted that cell pH does not fall below the value at which gluconeogenesis from lactate is specifically inhibited by acidosis (Iles *et al.*, 1977). However, it is possible that under hypoxic conditions the usual assumption of the 5,5'-dimethyloxazolidine-2,4-dione method, namely markedly less permeability of the plasma membrane to 5,5-dimethyloxazolidine-2,4-dione ions rather than undissociated 5,5'-dimethyloxazolidine-2,4-dione, might break down. In this case the intracellular pH falls calculated at low flow rates are likely to be underestimates. Nevertheless the magnitude of such errors would be diminished by any fall in membrane potential due to ischaemia (Roos, 1965; Cohen & Iles, 1975).

Table 1 shows that the 2:1 stoicheiometry between

lactate uptake and glucose output disappears at low flow rates, more glucose being produced than expected from the lactate uptake. This effect may be due to NAD^+ depletion because of hypoxia and consequent failure of lactate conversion into pyruvate. Presumably at low flow rates glucogenic precursors that do not require NAD^+ become relatively more important as glucogenic substrates. The observation that raising the perfusate lactate concentration at low flow rates increases lactate uptake but not glucose output suggests that mass-action effects allow lactate to compete more successfully for the available NAD^+ and endogenous amino acids may be partially displaced as glucogenic precursors by simple competition; alternatively, more lactate may be oxidized. The rise in the O_2 uptake/glucose output ratio at low flow rates suggests that the gluconeogenic pathway becomes a progressively relatively less favoured site of utilization of available ATP. However, the possibility that ATP-consuming substrate cycles in the gluconeogenesis pathway become more active at low flow rates cannot be excluded; for example, the phosphofructokinase–fructose bisphosphatase cycle could be stimulated by the fall in ATP concentration observed.

The fall of hepatic ATP content and rise in those of ADP and AMP caused by total ischaemia are well documented (Hems & Brosnan, 1970; Brosnan *et al.*, 1970). The present work shows that ATP concentration during relative ischaemia (13% of the normal flow rate) is better maintained than O_2 uptake and gluconeogenesis, decreasing by only 42% when the last two have fallen by 62 and 66% respectively, and it is not clear from the present results what factors are limiting gluconeogenesis at this extent of diminution of flow rate. The observations with increased concentration of lactate (4mM) appear to rule out substrate limitation. As pointed out above, the observed fall in intracellular pH is unlikely to be sufficient to account for inhibition of gluconeogenesis. It seems more probable that compartmentation of ATP makes simple comparison of decreases in ATP concentration and gluconeogenesis invalid. Two of the six molecules of ATP needed per molecule of glucose synthesized are utilized within the mitochondria; since mitochondrial volume is only a minor fraction (10–20%) of the total cell volume (Williamson, 1969), substantial falls in mitochondrial ATP concentration could easily be obscured in measurements of whole-cell ATP.

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APPENDIX

Model of Relation of Substrate Uptake to Flow in Hepatic Sinusoids

Let L be the lactate concentration at any point along the sinusoid, L_p and L_v the portal- and hepatic-venous lactate concentrations, t the time from entry into the sinusoid, U the overall lactate uptake during passage through the sinusoid, R the flow rate and T the transit time through the sinusoid. Assume that lactate uptake rate at any point in the sinusoid is proportional to L, that perfect mixing occurs in a lateral direction in the sinusoid and, for the moment, that all cells along the sinusoid have the same intrinsic capacity for lactate uptake.

With these assumptions, the decrease in L sustained in any small element of perfusate in the sinusoid during a period of time equal to the total transit time

would be the same whether that element was static or actually moving through the sinusoid. It follows that the decrease in L in a volume of blood V equal to that of the sinusoid may be considered by regarding it as entering the sinusoid instantaneously, remaining static for a period equal to the transit time and then instantaneously leaving the sinusoid. Considering this model, the amount of lactate leaving the sinusoid by entry into the cells is $-V\mathrm{d}L$ and:

$$-V\mathrm{d}L = KL\mathrm{d}t$$

(when K is a constant compounded of surface area and 'affinity' of the hepatocyte for lactate)

Thus: $L = L_p e^{-(K/V)t}$

But: $T = V/R$

and, when $t = T$, $L = L_v$

Thus: $L_v = L_p e^{-K/R}$

or

$$\ln\left(\frac{L_p}{L_v}\right) = \frac{K}{R} \quad (1)$$

From the Fick principle we have:

$$L_v = L_p - U/R$$

Substituting in eqn. (1) we obtain:

$$U = RL_p(1 - e^{-K/R}) \quad (2)$$

In the case where K varies along the sinusoid because of varying 'affinity' of the cells for lactate it is simple to show, by dividing the sinusoid into segments of different affinity and applying the above reasoning iteratively, that the new eqn. (1) differs only in the value of K, the slope of a plot of $\ln(L_p/L_v)$ against $1/R$. Thus variations in 'affinity' along the sinusoid do not affect the qualitative predictions of the model.