Hepatic Redox State and Gluconeogenesis from Lactate *in vivo* in the Rat

By RICHARD A. HAWKINS,* C. ROGER S. HOUGHTON† and DERMOT H. WILLIAMSON
Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary,
Oxford OX2 6HE, U.K.

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1. To examine the role of the hepatic redox state on the rate of gluconeogenesis the effects of sodium crotonate injection (6mmol/kg body wt.) on rat liver metabolite concentrations and gluconeogenesis from lactate were studied *in vivo*. 2. Crotonate caused a marked oxidation of cytoplasmic and mitochondrial redox couples; decreases were observed in the ratios of [lactate]/[pyruvate], [glycerol 3-phosphate]/[dihydroxyacetone phosphate], [hydroxybutyrate]/[acetoacetate] and measured $[NAD^+]/[NADH]$. 3. Increases occurred in the liver concentrations of all gluconeogenic intermediates from pyruvate through to glucose 6-phosphate, but there was no change in lactate concentration. 4. To determine whether gluconeogenesis from lactate was altered by the more-oxidized hepatic redox state L-[2- ^{14}C]lactic acid was infused into the inferior vena cava (50 μ mol/min per kg body wt.) and the incorporation of radioactivity into blood glucose was measured. 5. Administration of crotonate transiently decreased the rate of lactate incorporation into glucose but within a few minutes the rate of incorporation returned to that of the controls. 6. The results indicate that in these experiments alteration of the NAD^+ – $NADH$ systems of cytoplasm and mitochondria to a more-oxidized state did not change the rate of gluconeogenesis.

Under conditions where gluconeogenesis *in vivo* occurs at a high rate (i.e. starvation or diabetes) the cytoplasmic redox state is more reduced (Hohorst *et al.*, 1961; Wieland & Löffler, 1963). It has been suggested that a lowering of the cytoplasmic $[NAD^+]/[NADH]$ ratio may stimulate gluconeogenesis at the glyceraldehyde 3-phosphate dehydrogenase step (Williamson *et al.*, 1966; Williamson *et al.*, 1967). Evidence against this suggestion is the finding that gluconeogenesis from pyruvate in isolated perfused liver is as rapid as that from lactate (Ross *et al.*, 1966) in spite of the widely differing [lactate]/[pyruvate] ratios. Recent measurements of metabolite concentrations in rat liver under a variety of situations *in vivo* suggest that the redox state of the cytoplasmic NAD couple is controlled via the phosphorylation state of the adenine nucleotide system (Veech *et al.*, 1970; Stubbs *et al.*, 1972). However, although many of the earlier views have been modified (see, e.g., Williamson, 1971), it is still open whether the more-reduced cytoplasmic redox state is a controlling factor in gluconeogenesis or whether it merely reflects the

greater energy requirement in this situation (Krebs, 1971).

To provide further information on this question the rate of gluconeogenesis from L-[2- ^{14}C]lactic acid has been measured *in vivo* in normal starved rats and starved rats in which the hepatic redox state was made more oxidized by the injection of crotonate. Comparison of the hepatic metabolite patterns and the rates of conversion of lactate into glucose in the two situations indicate that an acute and sustained change to a more-oxidized hepatic redox state causes only a transient decrease in the rate of gluconeogenesis from lactate, with a rapid return to the control rate.

Materials and Methods

Rats

Male rats of the Wistar strain weighing about 200 g and starved for 48 h were used.

Chemicals and enzymes

Sodium [2- ^{14}C]pyruvate (15mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. It was reduced to L-[2- ^{14}C]lactate with $NADH$ and lactate dehydrogenase, followed by isolation by paper chromatography (Krebs *et al.*,

* Present address: Section on Neurochemistry, National Institute of Mental Health, Saint Elizabeth's Hospital, Washington, D.C. 20032, U.S.A.

† Present address: Department of Biochemistry, Royal Prince Alfred Hospital, Camperdown, Sydney, N.S.W. 2050, Australia.

1966). Other biochemicals and enzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.

Determination of metabolites

The following metabolites were measured by standard enzymic methods: L-lactate, L-malate, L-glycerol 3-phosphate and pyruvate (Hohorst *et al.*, 1959); D-3-phosphoglycerate, D-2-phosphoglycerate and phosphoenolpyruvate (Czok & Eckert, 1963); glucose 6-phosphate (Hohorst, 1963); D-3-hydroxybutyrate and acetoacetate (Williamson *et al.*, 1962); NAD⁺ and NADH (Klingenberg, 1963*a,b*); ATP (Lamprecht & Trautschold, 1963); ADP and AMP (Adam, 1963); D-glyceraldehyde 3-phosphate, dihydroxyacetone phosphate and fructose 1,6-diphosphate (Bücher & Hohorst, 1963); 2-oxoglutarate (Bergmeyer & Bernt, 1963); aspartate (Pfleiderer, 1963); L-glutamate (Bernt & Bergmeyer, 1963).

Treatment of liver

Liver was removed from the rats within 10s after they had been killed by cervical dislocation. It was immediately freeze-clamped and metabolites were extracted with 0.75 M-HClO₄ as previously described (Williamson *et al.*, 1967).

Infusion of lactic acid and blood sampling

Cannulae were placed in the femoral artery and the inferior vena cava as previously described except that two catheters were placed in the inferior vena cava, one through each femoral vein (Hawkins *et al.*, 1971). Heparin (1000 units/kg body wt.) was administered intravenously 30min before infusion. L-[2-¹⁴C]lactic acid (44000 d.p.m./ μ mol) was infused into the inferior vena cava of unanaesthetized rats at a rate of 100 μ mol/min per kg body wt. for 2min to obtain a blood concentration between 1 and 2mM and at a rate of 50 μ mol/min per kg body wt. thereafter. The free acid was infused to avoid the possibility of alkalosis. Blood samples (0.2ml) were obtained from one of the femoral artery catheters and added to 2ml of 0.3 M-HClO₄. After removal of denatured protein and neutralization with KOH in the standard way the resulting supernatant fluid was used for metabolite determinations.

The second catheter (located in the inferior vena cava) was used for injection of NaCl or sodium crotonate.

Determination of glucose specific radioactivity

Neutral blood extract (1ml) was passed over a column (3cm $\times$ 1cm) of Amberlite MB-2 (the resin was previously gassed for 2h with CO₂ to prevent absorption of glucose on the resin) and eluted with

2ml of water. The radioactivity of a portion of the eluate was measured and the glucose concentration was determined enzymically in another portion. Lactate could not be detected in the eluate by the enzymic method used.

Determination of radioactivity

A Beckman LS200 liquid-scintillation counter and scintillator fluid consisting of 6–8g of 5-(4-biphenyl)-2-(4-*t*-butylphenyl)-1-oxa-3,4-diazole (butyl-PBD; CIBA Ltd., Duxford, Cambs., U.K.), 80g of naphthalene (Thorn Electronics, Tolworth, Surrey, U.K.), 600ml of toluene and 400ml of 2-methoxyethanol were used.

Results

Changes in the concentration of liver metabolites after crotonate administration

The administration of crotonate caused no appreciable change in [ATP] or [ADP], but there was a 50% increase ($P < 0.01$) in [AMP] (Table 1). The concentrations of all gluconeogenic intermediates from pyruvate through to glucose 6-phosphate were raised, although the changes in [pyruvate], [phosphoenolpyruvate], [2-phosphoglycerate] and [3-phosphoglycerate] were the most marked. There was no change in [lactate], one of the main precursors of glucose in the starved state.

Evidence that the administration of crotonate shifted the redox state of the liver to a more-oxidized position is provided by the decrease in the ratios of [lactate]/[pyruvate], [glycerol 3-phosphate]/[dihydroxyacetone phosphate], [3-hydroxybutyrate]/[acetoacetate], [glutamate]/[oxoglutarate] and an increase in measured [NAD⁺]/[NADH] ratio (Table 2) (for interpretation of changes, see Holzer *et al.*, 1956; Bücher & Klingenberg, 1958; Williamson *et al.*, 1967).

Measurements of the rates of gluconeogenesis in vivo

To determine whether the more-oxidized hepatic redox state had any influence on gluconeogenesis the rate of glucose synthesis from lactate in control and crotonate-treated rats was measured. For this purpose L-[2-¹⁴C]lactic acid was infused at a constant rate (after preliminary loading) into the rats and the increase in specific radioactivity of the glucose with time was measured. Any decrease in the rate of gluconeogenesis from lactate should be shown by a rise in the blood lactate concentration and a decline in the increase in specific radioactivity of glucose, and the converse should occur if the rate of gluconeogenesis increased. One disadvantage of this system is that it is not possible to differentiate between changes in hepatic and renal gluconeogenesis.

Table 1. *Liver metabolite concentrations after crotonate injection*

Crotonate (sodium salt; 6mmol/kg body wt.) was injected intraperitoneally 15 min before the rats were killed. Controls were injected with an equivalent volume of 0.15M-NaCl. All values are means ($\pm$ S.E.M.) expressed as μ mol/g fresh wt., with the number of observations indicated in parentheses. Values for crotonate-injected rats that are statistically different from those for saline-injected rats are indicated by: * $P < 0.01$. The oxaloacetate concentration was calculated from the formula:

$$[\text{Oxaloacetate}] = \frac{[\text{pyruvate}] \times [\text{malate}]}{[\text{lactate}]} \times \frac{K_{\text{MDH}}}{K_{\text{LDH}}}$$

where K_{MDH} and K_{LDH} are the equilibrium constants of lactate dehydrogenase and malate dehydrogenase respectively (Williamson *et al.*, 1967; Hohorst *et al.*, 1959). For other details see the Materials and Methods section.

Metabolite	Concn. of metabolite		[Crotonate-injected] [control]
	Control (saline-injected)	Crotonate- injected	
Lactate (8)	0.304 $\pm$ 0.022	0.348 $\pm$ 0.035	1.14
Pyruvate (8)	0.027 $\pm$ 0.003	0.045 $\pm$ 0.003*	1.67
2-Oxoglutarate (8)	0.082 $\pm$ 0.011	0.173 $\pm$ 0.045*	2.11
Glutamate (3)	3.12 $\pm$ 0.163	2.64 $\pm$ 0.277	0.85
Malate (8)	0.337 $\pm$ 0.046	0.293 $\pm$ 0.054	0.87
Aspartate (3)	0.727 $\pm$ 0.056	0.411 $\pm$ 0.047*	0.57
Oxaloacetate (calculated) (8)	0.008 $\pm$ 0.001	0.011 $\pm$ 0.003	1.38
Phosphoenolpyruvate (8)	0.079 $\pm$ 0.004	0.151 $\pm$ 0.014*	1.91
2-Phosphoglycerate (8)	0.027 $\pm$ 0.003	0.063 $\pm$ 0.024	2.33
3-Phosphoglycerate (8)	0.185 $\pm$ 0.010	0.324 $\pm$ 0.027*	1.75
Glyceraldehyde 3-phosphate (3)	0.002 $\pm$ 0.0002	0.003 $\pm$ 0.0005	1.50
Dihydroxyacetone phosphate (3)	0.019 $\pm$ 0.003	0.022 $\pm$ 0.003	1.16
Glycerol 3-phosphate (8)	0.239 $\pm$ 0.028	0.124 $\pm$ 0.011*	0.52
Fructose 1,6-diphosphate (3)	0.003 $\pm$ 0.0002	0.005 $\pm$ 0.001	1.67
Glucose 6-phosphate (8)	0.061 $\pm$ 0.006	0.086 $\pm$ 0.015	1.41
3-Hydroxybutyrate (8)	1.84 $\pm$ 0.07	1.38 $\pm$ 0.09	0.75
Acetoacetate (8)	0.755 $\pm$ 0.040	1.05 $\pm$ 0.05*	1.39
ATP (8)	2.33 $\pm$ 0.09	2.11 $\pm$ 0.07	0.91
ADP (8)	0.843 $\pm$ 0.031	0.838 $\pm$ 0.034	0.99
AMP (8)	0.214 $\pm$ 0.031	0.323 $\pm$ 0.022*	1.51
NAD ⁺ (8)	0.821 $\pm$ 0.010	0.837 $\pm$ 0.022	1.02
NADH (7)	0.166 $\pm$ 0.008	0.147 $\pm$ 0.007	0.89

Table 2. *Changes in ratios of metabolite couples in rat liver after crotonate administration*

The values are means ($\pm$ S.E.M.) of ratios individually calculated from the experiments contained in Table 1. The number of observations are indicated in parentheses.

Ratios	Control (saline-injected)	Crotonate-injected
[Lactate]	13.2 $\pm$ 2.5 (8)	7.9 $\pm$ 0.97 (8)
[Pyruvate]		
[Glycerol 3-phosphate]	10.9 $\pm$ 1.8 (3)	6.6 $\pm$ 1.4 (3)
[Dihydroxyacetone phosphate]		
[3-Hydroxybutyrate]	2.49 $\pm$ 0.11 (8)	1.34 $\pm$ 0.11 (8)
[Acetoacetate]		
[Glutamate]	33.0 $\pm$ 4.3 (3)	13.9 $\pm$ 2.0 (3)
[Oxoglutarate]		
[NAD ⁺]	5.01 $\pm$ 0.29 (7)	5.73 $\pm$ 0.38 (7)
[NADH]		

Table 3. *Effect of crotonate administration on blood metabolite concentrations and blood glucose specific radioactivities during L-[2-¹⁴C]lactic acid infusion*

Crotonate (6 mmol/kg body wt.) or an equivalent volume of 0.15 M-NaCl was injected intravenously at 12 min after the start of the infusion. The results are mean values ($\pm$ S.E.M.) with the number of observations indicated in parentheses. For other details see the Materials and Methods section.

Treatment	Time (min)	[Hydroxybutyrate] (mM)	[Acetoacetate] (mM)	[Hydroxybutyrate] / [Acetoacetate]	[Lactate] (mM)	[Glucose] (mM)	$10^{-3} \times$ Glucose specific radioactivity (d.p.m./ μ mol)
NaCl	5	1.71 $\pm$ 0.45 (3)	1.35 $\pm$ 0.33 (3)	1.30 $\pm$ 0.42 (3)	1.44 $\pm$ 0.13 (7)	4.63 $\pm$ 0.25 (7)	7.36 $\pm$ 0.81 (7)
	10	—	1.28 $\pm$ 0.32 (3)	—	1.47 $\pm$ 0.23 (7)	5.20 $\pm$ 0.37 (7)	8.94 $\pm$ 0.69 (7)
	15	1.42 $\pm$ 0.39 (3)	1.17 $\pm$ 0.35 (3)	1.22 $\pm$ 0.15 (3)	1.27 $\pm$ 0.13 (7)	5.20 $\pm$ 0.31 (7)	9.92 $\pm$ 0.55 (7)
	20	1.33 $\pm$ 0.30 (3)	1.12 $\pm$ 0.29 (3)	1.20 $\pm$ 0.13 (3)	1.18 $\pm$ 0.07 (7)	5.50 $\pm$ 0.33 (7)	11.03 $\pm$ 0.47 (7)
	25	1.28 $\pm$ 0.36 (3)	1.15 $\pm$ 0.30 (3)	1.12 $\pm$ 0.18 (3)	1.18 $\pm$ 0.06 (7)	5.87 $\pm$ 0.33 (7)	12.15 $\pm$ 0.56 (7)
	30	1.17 $\pm$ 0.31 (3)	1.10 $\pm$ 0.26 (3)	1.07 $\pm$ 0.16 (3)	1.28 $\pm$ 0.09 (7)	6.14 $\pm$ 0.33 (7)	13.24 $\pm$ 0.48 (7)
Crotonate	5	1.65 $\pm$ 0.30 (5)	1.37 $\pm$ 0.20 (5)	1.18 $\pm$ 0.09 (5)	1.43 $\pm$ 0.14 (8)	4.81 $\pm$ 0.25 (8)	7.74 $\pm$ 0.96 (8)
	10	1.41 $\pm$ 0.29 (5)	1.27 $\pm$ 0.22 (5)	1.11 $\pm$ 0.09 (5)	1.35 $\pm$ 0.11 (8)	5.18 $\pm$ 0.29 (8)	9.32 $\pm$ 0.93 (8)
	15	1.32 $\pm$ 0.26 (5)	1.44 $\pm$ 0.22 (5)	0.90 $\pm$ 0.05 (5)	1.73 $\pm$ 0.17 (8)	5.85 $\pm$ 0.33 (8)	9.54 $\pm$ 0.90 (8)
	20	1.03 $\pm$ 0.21 (5)	1.56 $\pm$ 0.31 (5)	0.67 $\pm$ 0.04 (5)	1.43 $\pm$ 0.17 (8)	6.04 $\pm$ 0.41 (8)	10.55 $\pm$ 0.96 (8)
	25	0.87 $\pm$ 0.24 (5)	1.22 $\pm$ 0.25 (5)	0.55 $\pm$ 0.04 (5)	1.22 $\pm$ 0.11 (8)	5.94 $\pm$ 0.36 (8)	11.86 $\pm$ 0.87 (8)
	30	0.76 $\pm$ 0.25 (5)	1.44 $\pm$ 0.34 (5)	0.54 $\pm$ 0.04 (5)	1.14 $\pm$ 0.13 (8)	6.05 $\pm$ 0.40 (8)	12.50 $\pm$ 0.88 (8)

Fig. 1. Specific radioactivities of blood glucose in saline-injected and crotonate-injected rats

The results are plotted from the values given in Table 3 (for other experimental details see the text). ●, Saline-injected rats; ○, crotonate-injected rats. The arrow indicates the time of the injection of saline or crotonate.

The rate of lactic acid infusion ($50 \mu\text{mol/min}$ per kg body wt.) was chosen so as to equal approximately the measured rates of gluconeogenesis in starved rats ($35\text{--}60 \mu\text{mol/min}$ per kg body wt.; Baker *et al.*, 1959; Depocas, 1959; Ashby *et al.*, 1965; Dunn *et al.*, 1969). Lactic acid infusion into control rats gave a blood [lactate] of 1.44 mM after 5 min, with a slow decline to 1.28 mM at 30 min (Table 3). This was accompanied by a small decrease in total ketone-body concentration and in the [3-hydroxybutyrate]/[acetoacetate] ratio (Table 3). Incorporation of L-[2- ^{14}C]lactate into glucose was most rapid during the first 5 min, as may be expected because of the high initial rate of infusion and delivery of lactate of relatively high specific radioactivity to the liver before equilibration of blood lactate with extracellular fluids. Between 5 and 30 min of infusion the increase of specific radioactivity of glucose was almost linear (Table 3; Fig. 1).

Intravenous injection of crotonate at 12 min after the start of the lactic acid infusion caused a marked and sustained decrease in the [3-hydroxybutyrate]/[acetoacetate] ratio in blood, as would be expected from the more-oxidized state of the hepatic mitochondrial $\text{NAD}^+ - \text{NADH}$ system (Table 3). Gluconeogenesis from lactate was only impaired transiently,

as judged from the increase in blood [lactate] at 15 min (Table 3) and small decrease in the rate of L-[2- ^{14}C]lactate incorporation into glucose (Table 3, Fig. 1). Within a few minutes synthesis of glucose from lactate was restored to the control rate, as shown by the decrease in blood lactate concentrations (Table 3) and resumption of L-[2- ^{14}C]lactate incorporation into glucose (Fig. 1).

Discussion

Administration of crotonate to starved rats causes a prompt shift of the redox state of the hepatic NAD couple in the direction of oxidation (Table 2). An explanation for this may be based on the following facts. It is known that succinate (Chance & Hollunger, 1960; Chance & Williams, 1955a,b) and long-chain fatty acids (Krebs & Hems, 1970) cause a decrease of the mitochondrial $[\text{NAD}^+]/[\text{NADH}]$ ratio in the liver. A common feature of oxidation of succinate and long-chain fatty acids is the major involvement of a dehydrogenation where a flavoprotein rather than NAD^+ is the primary electron acceptor. Further, unless long-chain fatty acids are available to the liver the mitochondrial $[\text{NAD}^+]/[\text{NADH}]$ ratio increases above the range of values found *in vivo* (Krebs & Hems, 1970). It is also relevant that in the liver of starved rats the main oxidative process is the formation of ketone bodies from long-chain fatty acids. Since one-half of the hydrogen atoms in this process reach the respiratory chain at the flavoprotein level, the flux of hydrogen atoms from NADH to the flavoprotein of the respiratory chain must be substantially decreased. This would account for a higher steady-state concentration of NADH. Crotonate competes as a substrate of oxidation with long-chain fatty acids, but being already unsaturated no flavoprotein-linked step is involved when crotonate is converted into ketone bodies. Thus when crotonate replaces long-chain fatty acids the flux of hydrogen atoms from NADH through the respiratory chain is no longer restricted, and this is expected to result in the observed shift of the redox state in the direction of oxidation. An alternative though less likely explanation is that owing to the ready reversibility of the flavoprotein-linked dehydrogenation step in fatty acid oxidation (Hauge, 1956), crotonyl-CoA is reduced to butyryl-CoA, with concomitant oxidation of reduced flavoprotein.

Some evidence for the postulate that crotonate competes for the β -oxidation complex is provided by the increase in hepatic [AMP] in the rats treated with crotonate (Table 1). The medium-chain acyl-CoA synthetase is located within the mitochondrial matrix (Aas & Bremer, 1968; Haddock *et al.*, 1970), whereas the ATP-specific long-chain fatty acyl-CoA synthetase is associated with the outer mitochondrial membrane (Norum *et al.*, 1966; Haddock *et al.*, 1970). In

addition, the inner membrane is essentially impermeable to AMP and the phosphorylation of AMP is a slow process in mitochondria (Pfaff *et al.*, 1969) because of the absence of adenylate kinase from the matrix (Heldt & Schwalbach, 1967). Thus a rapid conversion of crotonate into crotonyl-CoA within the mitochondrial matrix might be expected to cause a rise in [AMP]. Experiments on isolated mitochondria where incubation with octanoate caused a considerably greater rise in intramitochondrial [AMP] than incubation with oleate (Stucki *et al.*, 1972) further support this view.

The concentration of ketone bodies remained relatively unchanged after administration of the large load of crotonate (Table 1). Crotonate, unlike long-chain fatty acids, cannot be converted into triglyceride, and therefore one potential control on its entry into the β -oxidation pathway does not apply. Thus it would appear that the proportion of acetyl-CoA arising from β -oxidation that is channelled to ketogenesis is subject to fine control in the starved state.

It is well established that gluconeogenesis from lactate in the isolated perfused liver (Struck *et al.*, 1966; Herrera *et al.*, 1966; Williamson *et al.*, 1966) and in kidney-cortex slices (Krebs *et al.*, 1963, 1965) is stimulated by the presence of free fatty acids. Three possible explanations are available for the role of free fatty acids in promoting gluconeogenesis: (1) provision of reducing equivalents; (2) provision of ATP; (3) increase of the concentration of acetyl-CoA required as an activator of pyruvate carboxylase. There is no reason to believe that the raised concentration of acetyl-CoA in starvation is decreased on administration of crotonate, and in fact crotonyl-CoA is also an activator of pyruvate carboxylase (Scrutton *et al.*, 1969). Crotonate, however, has a marked effect on the cytosolic and mitochondrial redox states and the provision of reducing equivalents. Assuming that the metabolite changes induced by crotonate in the single-injection experiments (Table 1) were similar to those occurring in the constant-infusion experiments (Table 3), it would appear that a shift in the redox state of the NAD^+ -NADH system of the liver cell to a more-oxidized state does not effect the rate of gluconeogenesis from lactate. Thus, contrary to previous suggestions (Williamson *et al.*, 1966; Williamson *et al.*, 1967) maintenance of a reduced NAD^+ -NADH system is not necessarily a prerequisite for gluconeogenesis.

Other evidence suggests that it is a suitable balance between the concentrations of gluconeogenic intermediates and the redox state which is the main consideration in controlling gluconeogenesis from lactate. An increase in the state of reduction of the cytoplasmic $[\text{NAD}^+]/[\text{NADH}]$ ratio after administration of ethanol to dogs results in a decrease in the hepatic glucose output and this inhibition can be

reversed by simultaneous infusion of Methylene Blue, which oxidizes NADH to NAD^+ (Madison, 1968). The explanation for this finding is provided by studies on rat liver which suggest that ethanol exerts its inhibitory action on gluconeogenesis from lactate by lowering the cytoplasmic $[\text{NAD}^+]/[\text{NADH}]$ ratio and consequently decreasing the concentration of pyruvate available for pyruvate carboxylase (Krebs, 1968).

In the present experiments, the ratio of $[\text{NAD}^+]/[\text{NADH}]$ in both liver cell compartments increased, but as a consequence of the change in the cytoplasmic redox state the concentration of pyruvate also increased, and the rate of gluconeogenesis from lactate remained relatively unchanged. The transient decrease in the rate of gluconeogenesis on administration of crotonate (Fig. 1) can be interpreted as a temporary hold-up of carbon in certain intermediates, owing to alteration of the redox state. If it is accepted that both the position of the redox state of the NAD^+ -NADH system and the concentrations of key intermediates are important factors in the control of glucose synthesis, it is presumably the increases in the concentration of these intermediates (e.g. pyruvate and oxaloacetate) which allowed the rate of gluconeogenesis to proceed unimpaired in these experiments.

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