Gluconeogenesis by Isolated Guinea-Pig Liver Parenchymal Cells

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1. Guinea-pig hepatocytes were prepared by collagenase digestion of the perfused liver. 2. The highest rates of gluconeogenesis were obtained from fructose, followed by pyruvate, xylitol and lactate, glycerol and propionate in that order. Maximum rates of gluconeogenesis were attained at 6–10 mM substrate. 3. An initial 15-min lag period occurred during gluconeogenesis from lactate. This lag was abolished by preincubating the cells or by preincubation plus the addition of NH_4Cl or lysine. 4. The lactate/pyruvate and 3-hydroxybutyrate/acetoacetate ratios were increased during the lag and adjusted to values favouring rapid gluconeogenesis from lactate after 15 min. 5. The data suggest that the low glucose synthesis during the lag resulted from a limitation of the glutamate–aspartate shuttle and from the unusual redox state of the NAD^+ couple prevailing during this period. 6. At 0.1 mM, amino-oxyacetate, a transaminase inhibitor, decreased gluconeogenesis from lactate by 80%, but had a negligible effect on glucose production from pyruvate. Gluconeogenesis from lactate was also inhibited (20%) by 10 mM-DL-3-hydroxybutyrate.

The isolated rat liver parenchymal cell system developed by Berry & Friend (1969) has been used to study a variety of metabolic processes *in vitro* (Ingebretsen *et al.*, 1972; Ontko, 1972; Johnson *et al.*, 1972; Capuzzi *et al.*, 1974; Seglen, 1974; Wilson *et al.*, 1974; Berger & Hommes, 1974; Haynes *et al.*, 1974). These cells synthesize glucose from lactate and pyruvate at rates ranging from 20–50% of the rates reported for the perfused rat liver (Ingebretsen & Wagle, 1972; Veneziale & Lohmar, 1973; Tolbert *et al.*, 1973) and constitute a suitable and simple system in which to study the regulation of hepatic gluconeogenesis.

For many years the use of the rat as a model has dominated studies on the regulation of gluconeogenesis despite species differences in the intracellular distribution of a key gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (Nordlie & Lardy, 1963; see Hanson & Garber, 1972, for review). We have shown that the pattern of regulation of hepatic gluconeogenesis in a variety of mammals, as demonstrated by studies with the perfused organ, differs significantly from that postulated for rat liver (Arinze *et al.*, 1973). This difference is most marked in species such as the guinea pig, rabbit and cat (Söling *et al.*, 1970; Arinze & Hanson, 1973; Arinze *et al.*, 1973; Johnson *et al.*, 1973), which, unlike the rat, have substantial activity of hepatic mitochondrial phosphoenolpyruvate carboxykinase. The potential advantages of studying metabolic processes, in particular gluconeogenesis, in animal models which deviate from the rat has been stressed (Hanson, 1974). This paper describes features of gluconeogenesis by

guinea-pig liver parenchymal cells isolated by collagenase digestion of the perfused organ.

Material and Methods

Chemicals

Analytical enzymes and cofactors were obtained from Boehringer Mannheim Corp., New York, N.Y., U.S.A. Amino-oxyacetic acid was purchased from Eastman Kodak Co., Rochester, N.Y., U.S.A., DL-3-hydroxybutyric acid (Na^+ salt) from Calbiochem, Los Angeles, Calif., U.S.A.; adenosine 3':5'-cyclic monophosphate, 6-N,2-O-dibutryryl-adenosine 3':5'-cyclic monophosphate, and swine skin gelatin from the Sigma Chemical Co., St. Louis, Mo., U.S.A. Collagenase (CLS II 44 D001, 232 units/mg; 44 C270, 317 units/mg and 44 A060, 150 units/mg; 1 unit of collagenase is the amount that liberates amino acids from collagen equivalent in ninhydrin colour to 1 μmol of L-leucine in 18 h at pH 7.4 and 37°C) was purchased from Worthington Biochemical Corp., Freehold, N.J., U.S.A. Purified glucagon (lot no. 258-D30-138-4) was a gift from Dr. William Bromer of Lilly Research Laboratories, Indianapolis, Ind., U.S.A. All other chemicals were of reagent grade and were obtained from readily available sources.

Animals

Male guinea pigs (200–250 g) of the Hartley strain were purchased from the Camm Research Institute, Wayne, N.J., U.S.A., and fed *ad libitum* on a pelleted Purina laboratory chow. The animals had free access to water at all times. Unless otherwise indicated food was withdrawn for 46–50 h before all experiments.

Isolation of liver parenchymal cells

Livers were isolated as described previously (Arinze *et al.*, 1973; Jomain-Baum *et al.*, 1973) and perfused at 37°C in a non-recirculating fashion with Ca^{2+} -free Krebs-Ringer bicarbonate buffer (Umbreit *et al.*, 1964), pH 7.4, which was continuously bubbled with an $\text{O}_2 + \text{CO}_2$ mixture (95:5). The first 100 ml of the perfusate used to wash out the liver was discarded and the system was then switched to a recycling mode. Hepatocytes were prepared from the perfused liver by an adaptation of the method developed for rat liver by Berry & Friend (1969) as modified by Zahltén *et al.* (1973). The addition of glutamate, fumarate, glucose and pyruvate in the perfusion medium as suggested by Zahltén *et al.* (1973) was omitted. After an initial 15 min of perfusion, collagenase (50 mg) was added and the flow rate was raised to 80–90 ml for 1–3 min to swell the liver and flood the hepatic sinuses with enzyme. The flow rate was then decreased to 30–40 ml to prevent collapse of the liver due to hydrostatic pressure. The perfusate volume was 150 ml. Leakage from the liver was returned to the reservoir until excessive leakage made continued perfusion unnecessary. Digestion of the liver with collagenase was usually complete in 15–20 min. The soft liver was then carefully removed, placed in a petri-dish and after removal of non-hepatic tissue, was minced with scissors and suspended in 60–70 ml of oxygenated Ca^{2+} -free Krebs-Ringer bicarbonate buffer containing 1.5% (w/v) gelatin (wash medium). Gelatin has been shown to be a suitable substitute for albumin (Zahltén & Stratman, 1974). The suspension was then filtered twice through cheese-cloth and then centrifuged at 50 g for 2 min at room temperature. The pellet was washed four–five times and recovered by centrifugation at 50 g for 40 s. The final pellet was suspended in 30–50 ml of oxygenated washed medium.

The dry weight of cells was determined by mixing an equal volume of cell suspension and 10% (w/v) solution of trichloroacetic acid, centrifuging at 15000 g (10 min) and washing once with 1% trichloroacetic acid (Berry & Friend, 1969). The pellet was dried at 110°C overnight and its weight determined. The wet weight of the cells was obtained by difference after centrifuging a portion of the preparation at 15000 g (10 min). In most preparations the wet weight was 30–50 mg/ml and the mean ($\pm$ s.e. 32 preparations) wet wt./dry wt. ratio was 3.6 ± 0.1 . The overall yield of cells from the liver was 30–40%. About 90–95% of the cells excluded Trypan Blue (0.2%). Cells were used for experiments within 10 min after the final wash.

Incubation

The basic incubation medium was Krebs-Ringer bicarbonate buffer, pH 7.4, containing 1.5% (w/v) gelatin. Solutions of all substrates and additions were

made with this buffer except for glucagon which was dissolved in 0.9% NaCl. The buffer and all solutions were bubbled with the $\text{O}_2 + \text{CO}_2$ gas mixture. Incubations were carried out in either 25 ml polycarbonate or glass Erlenmeyer flasks in a total volume of 3 ml in a Dubnoff metabolic shaker at 90 strokes/min at 37°C. The cells, 15–25 mg wet wt., were added to initiate the reaction, the flasks were gassed with the $\text{O}_2 + \text{CO}_2$ gas mixture for 10–15 s and sealed with rubber stoppers. In some experiments the substrate was added last after preincubating the cells for 10 min in buffer. The flasks were gassed after each addition. All incubations were done in duplicate and stopped with 0.5 ml of 36% (w/v) HClO_4 , chilled in ice and then neutralized with 5 M- K_2CO_3 . The resultant precipitate was centrifuged at 2500 g for 5 min and glucose in the supernatant was measured enzymically by the method of Slein (1965). Endogenous glucose production was measured in the absence of added substrate and all data shown, unless otherwise indicated, represent glucose production minus endogenous rates. Acetoacetate, 3-hydroxybutyrate, lactate and pyruvate were measured as described (Arinze *et al.*, 1973).

Results and Discussion

Total glucose production from 10 mM-lactate by isolated guinea-pig hepatocytes incubated in our basal incubation medium was proportional to the amount of added cell material. This proportionality was observed in most cell preparations containing 30–50 mg wet wt. of cells/ml. Consequently the weight/volume relationship in all preparations was adjusted to this range. With 10 mM-pyruvate as substrate, glucose production was linear for at least 75 min. Our basal incubation medium contained 1.3 mM- CaCl_2 . In the absence of Ca^{2+} , very little glucose was formed from lactate (results not shown).

Previous studies with the perfused guinea-pig liver have usually measured gluconeogenesis from pyruvate or lactate only at 2 mM substrate concentration (Arinze *et al.*, 1973; Jomain-Baum *et al.*, 1973). It seemed important to establish the gluconeogenic capacity of isolated guinea-pig hepatocytes with respect to variation in substrate concentration and to compare rates of gluconeogenesis from a variety of substrates. The dose-response curves for the generally used substrates (Fig. 1) show that gluconeogenesis by the isolated hepatocytes was near maximum at 6 mM. No substantial increase in glucose formation was achieved by increasing the substrate concentration to 10 mM. Table 1 shows a comparison of gluconeogenic rates from a variety of substrates at apparently saturating substrate concentrations. Of the substrates tested, the highest rates of gluconeogenesis were obtained from fructose, followed by pyruvate, xylitol and lactate, glycerol and propionate in that order.

The rates of glucose formation from alanine, propionate and glycerol were about 50–60% of the rates obtained with xylitol and lactate. When a lactate-pyruvate mixture (lactate concn. 10mM) in the physiological ratio of 30:1 (Garber & Hanson, 1971) was used as substrate the rate of gluconeogenesis was 25–30% more than with 10mM-lactate alone and was similar to that from 10mM-pyruvate alone.

The rates of gluconeogenesis from 10mM-lactate and pyruvate in these hepatocytes are equivalent to 0.50–0.81 $\mu\text{mol/min per g}$ on a wet weight basis and should be compared with 1.0–1.5 $\mu\text{mol/min per g}$ of wet tissue reported for the perfused guinea-pig liver at similar substrate concentration (Söling *et al.*, 1970; Arinze *et al.*, 1973). At considerably lower (2mM) concentrations of

pyruvate but not of lactate, the isolated guinea-pig hepatocytes synthesized glucose at 1.5 $\mu\text{mol/min per g dry wt.}$ (0.42 $\mu\text{mol/min per g wet wt.}$) which is slightly greater than the rates obtained with the perfused guinea-pig liver at this substrate concentration (Arinze *et al.*, 1973; Ogata *et al.*, 1974). These data compare favourably with studies with rat hepatocytes where rates of gluconeogenesis vary from 20–50% of the rate in the perfused rat liver (Berry & Friend, 1969; Ingebretsen & Wagle, 1972; Seglen, 1974; Tolbert & Fain, 1974; Veneziale & Lohmar, 1973; Zahltén *et al.*, 1973, 1974). The highest rates of gluconeogenesis reported for isolated rat hepatocytes are those of Cornell *et al.* (1973) whose rates are about 80% of that by the perfused organ. To our knowledge there has been no previous study of gluconeogenesis in isolated guinea-pig hepatocytes.

The response of the isolated hepatocytes to known effectors of hepatic gluconeogenesis was examined

Fig. 1. Variation of gluconeogenesis with substrate concentrations

The results are means $\pm$ S.E.M. for five experiments. All incubations were carried out in duplicate at 37°C for 60 min. For each point the endogenous glucose formation was subtracted from that in the presence of pyruvate (○), lactate (●) or alanine (▲).

Table 1. Glucose production from various gluconeogenic substrates by isolated guinea-pig liver parenchymal cells

Incubations were carried out in duplicate at 37°C for 50 min. For details see the Materials and Methods section. Endogenous glucose formation (glucose formed in the absence of added substrate) was subtracted to give the results indicated. Values are means $\pm$ S.E.M. for the number of experiments in parentheses.

Substrate added	Substrate concn. ...	Glucose formation ($\mu\text{mol/min per g dry wt.}$)	
		5mM	10mM
Fructose (7)		3.29 $\pm$ 0.25	3.56 $\pm$ 0.31
Glycerol (6)		1.15 $\pm$ 0.15	1.31 $\pm$ 0.22
Propionate (4)		0.97 $\pm$ 0.12	1.00 $\pm$ 0.05
Xylitol (8)		2.09 $\pm$ 0.16	2.12 $\pm$ 0.11
Alanine (3)		—	1.08 $\pm$ 0.10
Pyruvate (11)		—	2.56 $\pm$ 0.13
Lactate (12)		—	2.10 $\pm$ 0.10

Table 2. Effect of DL-3-hydroxybutyrate and amino-oxyacetate on gluconeogenesis from lactate and pyruvate by guinea-pig hepatocytes

Incubations were carried out for 60 min in duplicate as described in the Materials and Methods section. The concentration of lactate or pyruvate was 10mM. DL-3-Hydroxybutyrate and amino-oxyacetate were added to give the final concentrations indicated. In the absence of substrate these compounds did not appreciably change endogenous glucose production which was 0.54 $\pm$ 0.16 in their absence and 0.62 $\pm$ 0.21 and 0.64 $\pm$ 0.18 $\mu\text{mol/min per g dry wt.}$ respectively for incubations with 3-hydroxybutyrate (20mM) and amino-oxyacetate (0.2mM). Rates for glucose production are given as means $\pm$ S.E.M. for four experiments.

Addition (mM)	Lactate		Pyruvate	
	($\mu\text{mol/min per g dry wt.}$) (% of control)		($\mu\text{mol/min per g dry wt.}$) (% of control)	
None	2.08 $\pm$ 0.13	100	2.54 $\pm$ 0.26	100
3-Hydroxybutyrate (10)	1.72 $\pm$ 0.19	83	3.00 $\pm$ 0.29	118
3-Hydroxybutyrate (20)	1.52 $\pm$ 0.23	73	3.27 $\pm$ 0.53	129
Amino-oxyacetate (0.1)	0.40 $\pm$ 0.14	19	2.38 $\pm$ 0.22	94
Amino-oxyacetate (0.2)	0.30 $\pm$ 0.08	14	2.21 $\pm$ 0.26	87

by investigating the effect of amino-oxyacetate, an aminotransferase inhibitor (Hopper & Segal, 1962), and 3-hydroxybutyrate on gluconeogenesis from lactate and pyruvate. At 0.1 mM, amino-oxyacetate inhibited glucose formation from 10 mM-lactate by 80% and had negligible effect on gluconeogenesis from pyruvate (Table 2). The data are consistent with the known effects of amino-oxyacetate in the perfused liver (Arinze *et al.*, 1973) and with the established pathways of hepatic gluconeogenesis from these substrates (Walter *et al.*, 1966). However, the isolated hepatocytes appear to be more sensitive to inhibition by amino-oxyacetate than the perfused liver where inhibition by similar concentrations of amino-oxyacetate is about 60% (Arinze *et al.*, 1973). The increased sensitivity of the hepatocytes to amino-oxyacetate as opposed to the perfused guinea-pig liver is perhaps a result of the redox conditions during the incubation since a contributing factor to the overall inhibitory effect of amino-oxyacetate on gluconeogenesis is the suppression of NADH reoxidation (Arinze *et al.*, 1973). 3-Hydroxybutyrate,

Fig. 2. Sensitivity of isolated guinea-pig hepatocytes to glucagon, cyclic AMP and dibutyryl cyclic AMP

Parenchymal cells were isolated from livers of fed animals as described in the Materials and Methods section and incubated without added substrates for 20 or 30 min at 37°C (control, □), in the absence or the presence of glucagon (0.87 μM; 3 μg/ml; ▨), adenosine 3':5'-cyclic monophosphate (■), and 6-N,2'-O-dibutyryl adenosine 3':5'-cyclic monophosphate (▩). The cyclic nucleotides were added each at 0.1 mM. The glucose content of the cells at zero time has been subtracted. Therefore values given represent glucose released as a result of incubation. The results are the means ± S.E.M. for five experiments.

Table 3. Effect of various concentrations of NH_4Cl or lysine on gluconeogenesis from lactate by guinea-pig hepatocytes

Incubations were carried out for 60 min in duplicate as described in the Materials and Methods section. The concentration of lactate was 10 mM. NH_4Cl and lysine were added to give the final concentrations indicated. Appropriate controls were included for both additions and glucose formation in the absence of lactate subtracted. Values given are means ± S.E.M. for four experiments. The symbols* and † indicate significant differences from control: P values <0.001 and <0.05 respectively (Student's t test).

Concn. of NH_4Cl or lysine (mM)	Glucose formation (μmol/min per g dry wt.) in the presence of:	
	NH_4Cl	Lysine
0	1.72 ± 0.03	
1	2.15 ± 0.09*	2.22 ± 0.09*
2	1.99 ± 0.10†	2.35 ± 0.11
4	2.20 ± 0.12	2.50 ± 0.15
6	2.00 ± 0.15	2.34 ± 0.27

which stimulates gluconeogenesis in rat liver (Arinze *et al.*, 1973; Zahltén *et al.*, 1973), inhibits gluconeogenesis from lactate in isolated guinea-pig hepatocytes. This inhibition is comparable with that seen in the perfused guinea-pig liver and reflects the indirect inhibition of mitochondrial phosphoenolpyruvate formation by excess of mitochondrially generated NADH (Garber & Hanson, 1971; Arinze *et al.*, 1973; Bryla, 1974). As in the perfused liver, 3-hydroxybutyrate slightly increased gluconeogenesis from pyruvate.

Because Söling *et al.* (1970) reported a lack of effect of glucagon on hepatic gluconeogenesis in the perfused guinea-pig liver, the hormonal sensitivity of the isolated hepatocytes was examined in parenchymal cells isolated from livers of fed animals. Fig. 2 shows that such cells, incubated without substrates, released increased amounts of glucose into the incubation medium in the presence of glucagon, cyclic AMP or dibutyryl cyclic AMP. With 0.87 μM-glucagon, 40% more glucose was found in the medium after 20 min of incubation. At equimolar concentration (0.1 mM) both cyclic AMP and dibutyryl cyclic AMP increased glucose release to about the same extent after 20 min of incubation. The cyclic nucleotide-induced glucose formation after 30 min was usually greater in the presence of dibutyryl cyclic AMP than in the presence of cyclic AMP (Fig. 2). This may reflect a more rapid metabolism of cyclic AMP. Since these cells were prepared from livers taken from fed animals where endogenous glycogen is generally high, the glucose formation depicted in Fig. 2 reflects predominantly glycogenolysis.

An important feature of glucose synthesis by these hepatocytes was the consistently observed lag

Fig. 3 Effect of preincubation, NH₄Cl and L-(+)-lysine on the time-course of glucose formation from lactate by isolated guinea-pig hepatocytes

Cells were preincubated with buffer for 10min before the addition of lactate (10mM) at the points shown by the arrows. (a) Glucose formation from added lactate minus endogenous glucose formation for preincubated cells (●) or for incubations where cells and substrates were added together at the start of the incubation (Δ). For the preincubation experiments in (a), the zero time on the chart represents the point of addition of lactate. (b) and (c) show control incubations (○, □). Other additions were: ●, 10mM-lactate in (b) and (c); ■, 10mM-lactate plus 4mM-NH₄Cl in (b), and 10mM-lactate plus 1mM-lysine in (c). All incubations were conducted in duplicate at 37°C.

Table 4. Metabolite changes in cell suspension during 1 h of incubation in the presence and the absence of lactate

Cells were incubated in duplicate in the absence or presence of 10mM-lactate. Incubations were stopped at timed intervals as indicated. The zero time value is used to designate unincubated cells. Values are means ± s.e.m. for five–seven experiments or for three experiments where the s.e.m. are not indicated

Time of incubation (min)	Addition of lactate (10mM)	Metabolite changes (μmol/g dry wt.)					3-Hydroxy-butyrate/ acetoacetate
		Pyruvate	Lactate	Acetoacetate	3-Hydroxy-butyrate	Lactate/ pyruvate	
0	—	1.15 ± 0.06	19.44 ± 2.75	6.10 ± 1.11	2.93 ± 0.98	17.0 ± 2.4	0.47 ± 0.10
8	—	2.50 ± 0.26	29.44 ± 2.96	15.64 ± 2.86	3.85 ± 1.29	12.3 ± 1.5	0.25 ± 0.06
	+	22.67 ± 2.15		10.74 ± 1.47	2.68 ± 0.81		0.25 ± 0.06
15	—	2.34 ± 0.23	27.39 ± 2.86	18.40 ± 3.47	3.24 ± 0.84	12.3 ± 1.5	0.17 ± 0.02
	+	38.90 ± 3.47		13.05 ± 1.68	2.74 ± 0.90		0.18 ± 0.06
30	—	2.37 ± 0.43	21.24 ± 1.16	22.26 ± 4.87	1.74 ± 0.24	10.1 ± 1.7	0.08 ± 0.005
	+	77.66 ± 11.06		17.76 ± 3.69	2.83 ± 0.57		0.16 ± 0.02
45	—	2.58 ± 0.29	29.60 ± 2.70	26.09	2.57	10.6 ± 0.7	0.11
	+	81.15 ± 12.70		21.00 ± 4.14	3.02 ± 1.78		0.13 ± 0.07
60	—	3.38 ± 0.72	26.39 ± 2.78	24.32 ± 1.82	2.93 ± 0.66	8.6 ± 1.0	0.12 ± 0.02
	+	92.60 ± 14.58		26.12 ± 3.40	4.68 ± 1.65		0.19 ± 0.06

associated with gluconeogenesis from lactate, a phenomenon that was not seen when pyruvate was used as substrate. Zahlten *et al.* (1974; see also Cornell *et al.*, 1973) have shown that NH₄Cl increased glucose production in rat hepatocytes. Although in their experiments a lag was not always obvious, they suggest that NH₄Cl acts as an amino group donor for the synthesis of glutamate (presumably via glutamate

dehydrogenase) required for the glutamate–aspartate shuttle (Walter *et al.*, 1966). Indeed when NH₄Cl and lysine, which has also been shown to increase glucose production in rat hepatocytes (Cornell *et al.*, 1973; Tolbert & Fain, 1974) were included in our incubation system, they stimulated gluconeogenesis from lactate maximally in the range of 1–4mM (Table 3). When maximally effective concentrations of these

compounds were tested in time-course studies with lactate as substrate, the lag-time was not appreciably altered although they subsequently increased glucose production. In other experiments, however, where the cells were first preincubated in buffer, and lactate was then added after 10 min of incubation, it was clear that preincubation alone (Fig. 3a) or preincubation plus NH_4Cl or lysine (Figs. 3b,c) effectively eliminated the lag period associated with glucose formation from lactate. Glucose production was consistently higher at 15 min if the cells were preincubated than if they were not (Fig. 3a). The data support the idea that prior metabolism (preincubation) was necessary either to build up the concentration of endogenous metabolite(s), presumably glutamate and 2-oxoglutarate required for the glutamate-aspartate shuttle, or to adjust the redox state of the NAD^+ system to conditions more favourable for gluconeogenesis from lactate.

If the oxidation-reduction state of the NAD^+ couple were more in the direction of reduction at the start of the incubation, then the addition of lactate and its subsequent metabolism would further increase the concentration of reducing equivalents within the cells. This excess of reducing power could become limiting to gluconeogenesis from lactate. This seems possible since no lag was associated with gluconeogenesis from pyruvate. Table 4 shows the changes in the concentrations of metabolites (in cells plus medium) of the lactate and 3-hydroxybutyrate dehydrogenase systems at selected intervals during the usual 1 h of incubation. In incubations with 10 mM-lactate, this metabolite was not measured since the bulk of the lactate is likely to be extracellular. In incubations without substrate (equivalent to preincubation) the concentrations of pyruvate and acetoacetate were generally lower in the first 15 min than thereafter. Although not marked, the concentrations of lactate and especially 3-hydroxybutyrate in such flasks tended to fall as the incubation progressed. Consequently, both the ratios of lactate/pyruvate and 3-hydroxybutyrate/acetoacetate were much higher at the start of the incubation than at 30–60 min, indicating relatively reduced redox states of the NAD^+ couple in the cytoplasmic and mitochondrial compartments respectively. It is significant that the high ratios were observed at the crucial 15 min period associated with low glucose production from lactate. Both ratios remained relatively unchanged after 15 min during which time glucose production from lactate was rapidly accelerated (see Fig. 3).

While these studies were in progress, Cornell *et al.* (1974) reported a similar lag period in glucose synthesis from lactate by isolated rat liver cells which was significantly shortened by the addition of NH_4Cl , lysine and other amino acids. In their experiments, however, the lag phase was considerably longer (40 min) than in our studies but appeared to lead to a

similar decrease in the lactate/pyruvate ratio. The increase in gluconeogenesis in isolated liver cells caused by NH_4Cl may occur by the mechanism proposed by Zahlten *et al.* (1974). However, since the infusion of NH_4Cl into the perfused guinea-pig liver (Willms *et al.*, 1970) or its injection into rats (Williamson *et al.*, 1967) changes the mitochondrial redox state towards oxidation, the NH_4Cl -induced increase in glucose production in isolated hepatocytes may also be interpreted as resulting from the accompanying shift in the redox state. These considerations support the contention that the lag period associated with gluconeogenesis from lactate, and perhaps the lower rates of gluconeogenesis obtained with lactate versus pyruvate as substrate in hepatocytes reflect, in part, the oxidation-reduction conditions associated with the hepatocyte system.

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