Gluconeogenesis and the protection of hepatic intracellular pH during diabetic ketoacidosis in rats

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Studies were made of the mechanism whereby hepatic gluconeogenesis is increased in diabetic ketoacidosis (DKA) despite evidence *in vitro* of inhibition of gluconeogenesis by systemic acidosis. In perfused livers taken from normal rats, marked inhibition of lactate uptake and glucose output was achieved by simulation of metabolic acidosis in the perfusate. In perfused livers obtained from animals with DKA, lactate uptake and glucose output were greater than in normal perfused liver at all values of perfusate pH, and it was not possible to demonstrate significant inhibition of gluconeogenesis from lactate by perfusate acidosis. Varying severity of acidosis was induced in rats by (a) HCl infusion, (b) NH₄Cl ingestion or (c) experimental DKA. Hepatic intracellular pH (pH_i) was measured *in vivo* by ³¹P-n.m.r. spectroscopy. Whereas at the severer degrees of systemic acidosis marked falls in hepatic pH_i were seen in the HCl- and NH₄Cl-treated animals, little fall was seen in rats with DKA. The protection of hepatic pH_i in rats with DKA was not due to differences in respiratory compensation compared with the other groups. It is suggested that this protection of hepatic pH_i in DKA may be responsible for the failure of acidotic inhibition of gluconeogenesis from lactate. Possible reasons for pH_i protection in DKA are considered. There is no difference in hepatic energy status as assessed *in vivo* by ATP/P_i ratios between control, DKA and NH₄Cl-treated rats. DKA rats show a striking decrease in hepatic glycerophosphoethanolamine content.

INTRODUCTION

Under conditions simulating severe uncompensated metabolic acidosis, gluconeogenesis from lactate in the perfused rat liver is inhibited, provided that the lactate concentration in the perfusate exceeds 2 mM [1–4]. This inhibition seen *in vitro* is not consistent with the situation in clinical diabetic ketoacidosis (DKA), where, despite the systemic acidosis, hepatic gluconeogenesis is increased [5,6] and lactic acidosis, though a recognized accompaniment of DKA, is relatively uncommon [7]. This apparent anomaly gave rise to the studies reported here.

Livers taken from animals made diabetic with alloxan or streptozotocin, and perfused with a variety of gluconeogenic substrates, including lactate, demonstrate increased rates of gluconeogenesis compared with livers from non-diabetic animals [8,9]. In all of these studies the livers were perfused under normal acid–base conditions, i.e. at pH 7.4. However, it is unknown whether, under extracellular conditions of pH and ketone-body concentration simulating those seen in DKA, stimulation of gluconeogenesis is still observed in livers from diabetic rats, despite the acidosis and ketosis. The first objective of the present study was therefore to determine rates of gluconeogenesis from lactate in livers from diabetic rats perfused at low pH, with or without high 3-hydroxybutyrate concentration. We demonstrate that gluconeogenesis from lactate in livers taken from diabetic rats is resistant to inhibition by perfusate acidosis.

The proximate cause of acidotic depression of gluconeogenesis from lactate appears to be lowering of hepatic

intracellular pH (pH_i) [2,3,10]. The metabolic block is located at a step between pyruvate and oxaloacetate [3]. The second objective of this study is therefore to determine whether the expected fall in hepatic pH_i in DKA does in fact occur *in vivo* and to compare the effect of DKA on hepatic pH_i *in vivo* with that resulting from other forms of systemic acidosis. ³¹P-n.m.r. spectroscopy was used to measure hepatic pH_i *in vivo* in rats with acidosis caused by administration of NH₄Cl or HCl, or by DKA. We find that, under conditions of severe systemic acidosis, hepatic pH_i is substantially less depressed in animals with DKA than in those animals with the other forms of metabolic acidosis. We also report measurements of hepatic ATP/P_i ratio derived from the same spectra as the pH_i values, and of adenine nucleotide and phosphodiester concentrations in extracts of livers obtained from animals similarly treated.

METHODS

Liver perfusions (groups 1 and 2)

Male Wistar rats (150–230 g) were used and divided into two groups. Group 1 animals were starved for 48 h before perfusion. Immediately before starvation, group 2 rats were anaesthetized with ether and given an intravenous injection of 80–100 mg of streptozotocin/kg in pH 4 citrate buffer. Then 48 h later, immediately before liver perfusion, rats were anaesthetized and samples of venous blood (1 ml) taken for measurement of pH, pCO₂, pO₂, 3-hydroxybutyrate, lactate and glucose.

Isolated rat liver perfusions were carried out at 37 °C

Abbreviations used: DKA, diabetic ketoacidosis; pH_i, intracellular pH; pH_e, perfusate pH; PCr, phosphocreatine.

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as previously described [11]. The perfusion medium consisted of 17% (w/v) bovine serum albumin (Fraction V) dissolved in Krebs bicarbonate buffer containing human erythrocytes (> 4 weeks post-donation) to give a packed cell volume of 17–20%. Perfusions were carried out on both control and diabetic rats at a flow rate of 7.5 ml/min per 100 g rat. Animals in groups 1 and 2 were divided into further subgroups [designated (a), (b) and (c)] according to whether the perfusate pH was adjusted (by manipulation of bicarbonate concentration while maintaining $p\text{CO}_2$ constant) to 7.4, 7.0 or 6.8; further groups of livers from control and diabetic rats were perfused at pH 7.0 with the addition of 10 mM-DL-3-hydroxybutyrate to the perfusate. All perfusion media contained 5 mM-L-lactate. Livers were perfused in the recirculating mode for 20 min, and then in the non-recirculating mode for a further 15 min. At 25, 30 and 35 min, portal and hepatic venous samples of perfusate were taken for the measurement of lactate uptake and glucose output, and the values at the three times were averaged. After the third pair of samples was taken, the liver was excised and weighed.

³¹P-n.m.r. studies *in vivo* of pH_i , P_i and adenine nucleotides (groups 3–6)

Male Wistar rats, starved for 48 h and weighing 160–225 g, were used. They were divided into four groups for study. In group 3, the control group, anaesthetized animals were either untreated or given an intravenous infusion of iso-osmotic saline (4–12 ml/kg body wt. over approx. 30 min). In group 4, animals were given 4–12 ml of NH_4Cl (1.87 M) by intragastric infusion. In group 5, animals received an intravenous infusion of 4–6 ml of HCl (0.3 M) at 0.05 ml/min (via an indwelling atrial cannula which had been implanted 2 days previously). All infusions were given 1–6 h before ³¹P-n.m.r. studies. Finally, to induce diabetes, some animals (Group 6) received an intravenous injection of streptozotocin (100 mg/kg body wt.) 48 h before the ³¹P-n.m.r. studies. Mean mixed venous 3-hydroxybutyrate concentration was 6.14 mM in animals whose mixed venous pH was less than 7.0, with a maximum value of 18.4 mM.

Samples of venous blood (1 ml) were taken from animals in groups 4–6 immediately before n.m.r. for the measurement of pH, $p\text{CO}_2$, $p\text{O}_2$ and blood 3-hydroxybutyrate and glucose.

Rats were then anaesthetized with Inactin [5-ethyl-5-(methylpropyl)-2-thiobarbiturate] at a dose of 100 mg/kg body wt. and secured in a custom-built n.m.r. probe. Rectal temperature was monitored and maintained close to 37 °C throughout the pre-n.m.r. procedures and during the time spent in the magnet.

N.m.r. experiments were performed at 8.46 T or 4.7 T with either a Bruker AM360 or WM200 wide-bore spectrometer operating in the Fourier Transform mode. An insulated surface coil (1.2 cm diameter) was used as both a transmitter and receiver coil; this was placed either directly on the surgically exposed liver [12] or over the assumed position of the underlying liver, just below the xiphoid cartilage. In the latter case the liver was 'massaged' through the abdominal muscle wall so that it hung away from the diaphragm.

Field homogeneity was optimized by using the proton signal from water within the liver, and the field was adjusted to bring this water signal on to resonance at a constant radio-frequency offset. This provides an effective

chemical-shift reference for ³¹P [13]. In the exposed-liver experiments, radio-frequency pulses of approx. 90° at the centre of the coil were used, with a pulse repetition interval of either 0.6 s or, for quantifiable spectra, 4 s. In the experiments using an externally arranged surface coil, spectra from predominantly liver tissue were obtained by increasing the pulse angle beyond 90° at the surface. Again, a pulse repetition interval of 0.6 s was adopted. The pulse width which gave a minimal phosphocreatine (PCr) resonance (PCr is absent from liver) was used, and probably represented a 180° pulse at the surface (see Fig. 1). Comparison of the liver and skeletal-muscle spectra in Fig. 1 indicates that, although a small PCr signal appears in the former, owing to contamination from neighbouring skeletal muscle, the ratio of PCr to P_i in the latter is such that this contamination has a negligible effect on the P_i signal from liver. Chemical shifts are expressed relative to a value for PCr of 0 p.p.m. When PCr was absent from the spectrum, the water proton resonance was used as the standard [13]. The data were processed with a 30 Hz line-broadening function. pH_i was determined from the pH-sensitive chemical shift of P_i [14]. Peak areas were determined by cutting and weighing of the peaks after drawing of the baseline by hand.

Fig. 1. ³¹P-n.m.r. spectra of abdominal skeletal muscle (upper trace) and liver (lower trace)

Key: GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; α , β and γ refer to the respective phosphate groups of ATP; PW, pulse width. Data were collected by using a surface coil placed on the abdomen just below the xiphoid. Each spectrum is the sum of 64 scans with a pulse interval of 0.6 s. Localization was achieved by alteration of pulse width as indicated.

³¹P-n.m.r. measurement of hepatic phosphates *in vitro*

Male Wistar rats of approx. 200 g were starved for 48 h. Streptozotocin (80 mg/kg body wt.) in citrate buffer (pH 4) was administered intravenously immediately before starvation to a separate group of rats. At the end of the starvation period, the rats were anaesthetized and samples of venous blood (1 ml) were taken for blood gas and metabolite measurement. The livers were then rapidly excised and freeze-clamped with aluminium tongs pre-cooled in liquid N₂. The frozen tissue was powdered under liquid N₂ and homogenized with approx. 2 vol. of 10% (w/v) pre-chilled (4 °C) HClO₄. Homogenates were centrifuged at 2400 g for 5 min and the supernatants decanted and stored at -70 °C. The samples were thawed to 4 °C, neutralized with triethanolamine chloride/KOH (1 M/2 M) buffer and centrifuged (2400 g). ³¹P-n.m.r. studies of the supernatants were performed at 81 MHz by using a standard 15 mm tube and a multi-nuclear broad-band Bruker probe. A 0.1 ml portion of PCr (100 mM) was added to a 5 ml neutralized liver extract to act both as a chemical-shift reference and as an absolute concentration standard; 0.25 ml of ²H₂O and approx. 10 mg of EDTA were also added, and the spectrometer was locked and the field shimmed by using both the ²H₂O and water signals. EDTA was used to chelate paramagnetic ions, which are abundant in the liver. Quantifiable spectra were accumulated by using 30° pulses with an 8 s repetition delay. Concentrations of phosphorus metabolites were calculated by comparison of their peak areas with the peak area of the PCr standard (approx. 2 mM). Peak areas were measured by

computer integration or by the 'cut-and-weigh' method, which gave similar results.

Chemical methods

Lactate, 3-hydroxybutyrate, acetoacetate and glucose were determined in HClO₄ extracts of blood or perfusate by using standard enzymic analyses [15]. pH, pCO₂ and bicarbonate were determined with an IL413 or 1304 blood-gas analyser.

Statistical methods

All results are means ± S.E.M. In the perfusion studies, rates of glucose output and lactate removal are means of the average rates in each individual experiment, calculated from the results at the three sample times referred to above. Where appropriate, significances of differences between means were calculated by Student's *t* test (unpaired, two-tailed).

In the plot of intrahepatic pH against extracellular pH (Fig. 2), linear fits were made to the data in each group. Analysis of co-variance by using a linear contrast between the DKA group and the other two groups was performed [16].

RESULTS**Perfusion studies**

Values of venous-blood pH, pCO₂, blood glucose, 3-hydroxybutyrate and lactate immediately before perfusion are shown in Table 1, together with perfusate pH (pH_e) and pCO₂ from the perfused livers of control and diabetic

Table 1. Pre-perfusion atrial-blood pH, pCO₂ and metabolite concentrations, and perfusate pH and pCO₂ from studies in normal and DKA rats

The left-hand set of data were obtained *in vivo* before preparation of livers for perfusion. The third column shows the nominal perfusion pH and the penultimate column the perfusion pH actually achieved. For simplicity, the nominal pH values are referred to in Table 2 and the text. Abbreviations: ND, no results; OHB, D-3-hydroxybutyrate.

Group no.	Condition	Pre-perfusion atrial-blood data <i>in vivo</i>						Portal (PV) and hepatic-venous (HV) perfusate pH and pCO ₂ after 20 min recirculation	
		Nominal perfusion pH	pH	pCO ₂ (kPa)	OHB (mM)	Glucose (mM)	Lactate (mM)	pH	pCO ₂ (kPa)
1(a)	Normal	7.4	ND	ND	1.62 ± 0.22 (n = 5)	5.70 ± 0.08 (n = 5)	1.18 ± 0.15 (n = 5)	PV 7.44 ± 0.01 HV 7.43 ± 0.1 (n = 6)	4.88 ± 0.14 4.97 ± 0.11 (n = 6)
1(b)	Normal	7.0	ND	ND	0.80 ± 0.4 (n = 5)	5.82 ± 0.41 (n = 5)	1.09 ± 0.33 (n = 5)	PV 6.99 ± 0.5 HV 6.98 ± 0.05 (n = 5)	5.42 ± 0.26 5.85 ± 0.31 (n = 5)
1(c)	Normal	6.8	ND	ND	1.24 ± 0.1 (n = 4)	4.11 ± 1.3 (n = 4)	1.08 ± 0.14 (n = 4)	PV 6.82 ± 0.03 HV 6.85 ± 0.03 (n = 4)	5.15 ± 0.28 5.36 ± 0.24 (n = 4)
2(a)	Diabetic	7.4	6.98 ± 0.17 (n = 5)	4.86 ± 0.39 (n = 5)	7.25 ± 1.11 (n = 5)	19.2 ± 1.29 (n = 5)	1.24 ± 0.19 (n = 5)	PV 7.41 ± 0.01 HV 7.47 ± 0.02 (n = 5)	4.79 ± 0.13 4.86 ± 0.14 (n = 5)
2(b)	Diabetic	7.0	6.85 ± 0.11 (n = 7)	6.04 ± 0.34 (n = 7)	8.13 ± 1.94 (n = 5)	26.3 ± 4.15 (n = 7)	1.08 ± 0.18 (n = 6)	PV 7.01 ± 0.02 HV 7.04 ± 0.02 (n = 7)	5.61 ± 0.24 5.69 ± 0.28 (n = 7)
2(c)	Diabetic	6.8	6.82 ± 0.08 (n = 5)	4.43 ± 0.39 (n = 5)	7.77 ± 0.33 (n = 8)	18.93 ± 0.95 (n = 8)	1.33 ± 0.12 (n = 8)	PV 6.82 ± 0.02 HV 6.86 ± 0.03 (n = 8)	5.20 ± 0.23 5.23 ± 0.19 (n = 8)

Table 2. Lactate uptake and glucose output in perfusions of livers from control and DKA rats at different perfusate pH values

Significant difference compared with perfusion at pH 7.4 in the same group is shown by * $P < 0.01$ or ** $P < 0.001$. The data shown in the diabetic subgroups are also compared with the corresponding subgroups from control (non-diabetic) rats ($\dagger P < 0.05$; $\ddagger P < 0.01$; $\ddagger\ddagger P < 0.001$). See the legend to Table 1 for further explanations of Groups.

	Subgroups	<i>n</i>	Nominal perfusate pH	Lactate uptake ($\mu\text{mol/min per 100 g body wt.}$)	Glucose output ($\mu\text{mol/min per 100 g body wt.}$)	Lactate uptake / Glucose output
Control	1(a)	6	7.4	7.26 ± 0.38	3.82 ± 0.2	1.9 ± 0.04
	1(b)	5	7.0	5.78 ± 0.92	$2.39 \pm 0.3^*$	2.42 ± 0.07
	1(c)	4	6.8	$4.16 \pm 0.28^{**}$	$2.29 \pm 0.36^*$	1.95 ± 0.29
DKA	2(a)	7	7.4	$10.62 \pm 1.11\dagger$	$6.56 \pm 0.29\dagger\dagger\dagger$	1.63 ± 0.15
	2(b)	7	7.0	$11.11 \pm 1.29\dagger$	$6.07 \pm 0.68\dagger\dagger$	1.77 ± 0.18
	2(c)	8	6.8	$8.74 \pm 0.59\dagger\dagger\dagger$	$5.82 \pm 0.35\dagger\dagger\dagger$	1.44 ± 0.06

animals. There is no significant difference among any of the variables in the pre-perfusion data from the diabetic rats later allocated to the three perfusion pH groups [2(a), 2(b), 2(c)]. The pH and $p\text{CO}_2$ values from the portal- and hepatic-venous perfusate show no difference between corresponding subgroups in groups 1 and 2. There is a tendency throughout for hepatic-venous pH to be slightly higher than in the portal inflow, despite a marginally higher $p\text{CO}_2$ in the former, a phenomenon previously noted [17] and attributed to the metabolism of lactate to neutral products.

Effect of acidosis on lactate uptake and gluconeogenesis (Table 2)

In the control group, the mean rate of uptake of lactate was progressively decreased by acidosis, and highly significantly so at pH 6.8. Hepatic glucose release showed similar changes. The ratio of lactate uptake to glucose output was similar in each subgroup at approx. 2:1, suggesting that lactate was the sole substrate for glucose synthesis. In the diabetic group, however, the mean rates of both lactate uptake and glucose release were slightly decreased by pH, but these effects were not significant. Compared with the control group, the rates of lactate removal and gluconeogenesis in the 'diabetic' perfusions were consistently and significantly higher at all pH values. The ratio of lactate uptake/glucose output in the livers from diabetic animals was less than 2:1 in all groups, suggesting that up to 25% of the glucose was derived from sources other than lactate. The liver-weight/body-weight ratios in the diabetic groups were approx. 13% greater than in the control groups.

Effect of (DL)-3-hydroxybutyrate on lactate uptake and gluconeogenesis

When 10 mM-3-hydroxybutyrate was added to perfusions at pH 7.0 of livers from control (non-diabetic) rats, mean lactate uptake was $6.29 \pm 0.61 \mu\text{mol/min per 100 g body wt.}$ and mean glucose output was $2.85 \pm 0.41 \mu\text{mol/min per 100 g}$ ($n = 4$). These means do not differ significantly from those obtained in perfusions at this pH in the absence of 3-hydroxybutyrate [see subgroup 1(b), Table 2]. When 10 mM-3-hydroxybutyrate was added to perfusions at pH 7.0 of livers from diabetic rats, mean lactate uptake and glucose output were

Fig. 2. ^{31}P -N.M.R. spectra obtained by using a coil placed directly on the liver surface in control (a) and DKA animals (b)

Key: PM, phosphomonoesters; PD, phosphodiesterase; UDPG, UDP-glucose. Each spectrum is obtained from 200 scans with a 4 s pulse interval.

9.74 ± 0.78 and 5.16 ± 0.67 $\mu\text{mol}/\text{min}$ per 100 g ($n = 6$) respectively. Again, these means are not significantly different from those obtained in perfusions of livers from diabetic rats in the absence of 3-hydroxybutyrate [see subgroup 2(b), Table 2].

³¹P-n.m.r. studies *in vivo*

Fig. 2 shows spectra for liver from a control and a diabetic rat; the spectra are almost identical, and also similar to the liver spectrum in Fig. 1. Fig. 3 shows the relationship of mixed venous pH (pH_e) to intracellular pH (pH_i) in the control rats (group 3) and in the three groups of animals made acidotic by NH_4Cl ingestion (group 4), by HCl infusion (group 5) or by the induction of DKA (group 6). In all rats in the DKA group, blood glucose exceeded 15 mM and blood 3-hydroxybutyrate exceeded 4 mM in samples taken immediately before n.m.r. studies. A wide range of pH_e was achieved in each group. In the acidotic animals, pH_i is similar at mild degrees of acidosis in all three groups. However at more severe degrees of systemic acidosis, pH_i remains much higher in the DKA group than in the NH_4Cl or HCl groups. Linear fits were made to the three sets of data, and the slope of the regression of pH_i on pH_e was significantly ($P < 0.02$) less in the DKA group (slope 0.225) than in the NH_4Cl (slope 0.765) and HCl (slope 1.02) groups. Analysis of covariance by using a linear contrast between the DKA and the other groups [16] was significant ($P < 0.006$).

Mean mixed venous pCO_2 in groups 4–6 was 5.61 ± 0.14 , 6.29 ± 0.27 and 6.4 ± 0.4 kPa respectively when $\text{pH}_e > 7.1$, and slightly lower (5.48 ± 0.23 , 5.18 ± 0.47 and 5.62 ± 0.37 kPa) when $\text{pH}_e < 7.1$, but there were no significant differences between the groups in either pH range.

Absolute quantification of nuclear magnetic resonances *in vivo* is difficult by using surface coils, principally because the volume from which signals are received is ill-defined. However, metabolite ratios can be accurately reproducibly measured provided that attention is paid to saturation. The 4 s pulse delay is sufficiently long to avoid partial saturation of resonances. The ATP/ P_i ratios in groups 3, 4 and 6 were 2.62 ± 0.61 ($n = 5$), 2.66 ± 0.17 ($n = 5$) and 2.39 ± 0.31 ($n = 7$) respectively. There are no significant differences among the groups. Most of the group 5 experiments were carried out with a pulse repetition rate of 0.6 s only, and the ATP/ P_i ratios for this group have not been calculated.

Right-atrial ('mixed venous') arterial and portal-venous pH and pCO_2

To examine whether the relationship between pH and pCO_2 in these three sampling sites varied between DKA animals and those with NH_4Cl -induced acidosis, groups of such animals were made severely acidotic, anaesthetized, and blood samples were rapidly obtained from the iliac artery and hepatic portal vein (through an abdominal incision) as well as from the pre-placed right arterial

Fig. 3. Relationship *in vivo* between intracellular pH (pH_i) and right-atrial blood pH (pH_e) in (a) group 4 (NH_4Cl acidosis) (Δ), (b) group 5 (acidosis induced by HCl infusion) ($\circ$) and (c) group 6 (DKA) ($\bullet$)

In (d) the data of (a), (b) and (c) are superimposed. $\blacklozenge$, data from the control animals (group 3) (the same data in each diagram); these control data have been excluded in the statistical analyses described in the text.

Table 3. P_i , adenine nucleotides, glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC) in hepatic extracts from control and DKA ratsResults are expressed as $\mu\text{mol/ml}$ of total tissue water (means $\pm$ S.E.M.): * $P < 0.001$.

		P_i	ADP	ATP	GPE	GPC	ATP/ P_i	Venous 3-hydroxybutyrate (mM)
Control	($n = 10$)	4.4 ± 0.12	0.6 ± 0.04	3.07 ± 0.22	2.77 ± 0.36	1.14 ± 0.11	0.77 ± 0.05	$0.32, 0.57$ ($n = 2$)
DKA	($n = 6$)	4.47 ± 0.2	0.7 ± 0.08	3.14 ± 0.016	$0.81 \pm 0.15^*$	1.19 ± 0.3	0.80 ± 0.03	4.6 ± 1.6

cannula. The mean difference between right-atrial pH (pH_e) and arterial pH (pH_a) was not significant between the groups (DKA: -0.014 ± 0.002 , $n = 6$; NH_4Cl : -0.024 ± 0.008 , $n = 5$). Nor was there a significant difference between the groups in the mean pH gradient from hepatic portal vein to right atrium (DKA: 0.003 ± 0.005 ; NH_4Cl : -0.005 ± 0.008). Mean pH_e did not differ significantly in the two groups (DKA group 6.87 ± 0.08 ; NH_4Cl group 6.98 ± 0.03). Nor was there any significant difference between the groups in mean pH_a (6.88 ± 0.08 and 7.00 ± 0.03 respectively) or portal-venous pH (6.87 ± 0.08 and 6.98 ± 0.03 respectively). There were also no significant differences between the groups in mixed-venous, arterial or portal-venous $p\text{CO}_2$.

³¹P-n.m.r. studies in liver extracts

Table 3 shows P_i , ATP, ADP and phosphodiester concentrations in control and DKA animals. Except for glycerylphosphoethanolamine, which is decreased in livers of diabetic animals to 30% of the control values ($P < 0.005$), there are no significant differences in these variables, nor in ATP/ P_i ratio, between the two groups. ADP was readily measurable in the extracts, but not in the spectra obtained *in vivo*, as previously [18]. On the basis of our previous work on perfused liver, we have assumed that the extract ATP concentrations reflect the concentration of 'n.m.r.-visible' ATP in the spectra recorded *in vivo* [18].

DISCUSSION

The results of the perfusion experiments confirm earlier work indicating that livers from diabetic rats have higher rates of gluconeogenesis from lactate [8,9]. The present study shows that livers from DKA rats are resistant to the inhibitory effect of acidosis on gluconeogenesis from lactate that is found in livers from normal rats. Both lactate uptake and glucose output are considerably higher in the diabetic group at all pH_e values.

The ratios of lactate uptake/glucose output are approximately stoichiometric in the non-diabetic perfusions, but are substantially less than 2 in the diabetic perfusions, suggesting glucose synthesis from non-lactate sources, e.g. endogenous amino acids, in the diabetic livers.

The choice of 5 mM as the concentration of lactate in the perfusate requires some discussion. Lactate entry into the hepatocyte occurs by two mechanisms [4,19–21]. The first is a stereospecific lactate transporter which is rapidly saturated at lactate levels greater than 2 mM. Transporter activity is stimulated by a fall in pH_e relative to pH_i [19,20], is inhibited by 3-hydroxybutyrate and

acetoacetate and is rate-limiting for lactate uptake in intact perfused liver from starved rats at low or physiologically normal lactate concentrations, i.e. below about 1.5 mM [22]. Stimulation of the transporter may be the reason why the inhibitory effect of acidosis on gluconeogenesis from lactate cannot be observed at these lower concentrations of lactate [23]. The second mechanism of entry is non-saturable, and is probably pH-dependent non-ionic diffusion [24]. The concentration of 5 mM-lactate was chosen so that the results should be substantially independent of effects of pH or 3-hydroxybutyrate on the transporter.

The addition of 3-hydroxybutyrate made no significant difference to lactate uptake or glucose output in perfusions at pH 7.0 of livers from either control rats or rats with DKA. The lack of effect of 3-hydroxybutyrate suggests that the conclusions about the resistance to acidotic suppression of gluconeogenesis obtained from perfusions without 3-hydroxybutyrate are unlikely to be affected by the presence of 3-hydroxybutyrate in concentrations of the order seen in clinical and experimental DKA.

Fig. 3 shows that the intracellular pH of the liver *in vivo* in DKA rats is not depressed to the same extent as in HCl- or NH_4Cl -treated rats for a given degree of extracellular acidosis below pH 7.0. We show here that this result is not likely to be due to differences between DKA and NH_4Cl -induced acidosis in the relationship between pH (or $p\text{CO}_2$) in 'mixed venous', arterial and portal-venous blood. It is therefore probable that the reason for the 'protection' of pH_i in DKA lies within the liver rather than in the external acid-base environment, and we have assumed in the following discussion that the same is true for the DKA/HCl comparison. This protection could explain why gluconeogenesis *in vivo* in DKA is not inhibited by the extracellular acidosis. If the 'protection' of hepatic pH_i seen in DKA *in vivo* persists into the perfused liver from DKA rats, this might also explain the failure of severe perfusate acidosis to inhibit gluconeogenesis from lactate. In baseline observations for a separate study of the effects of bicarbonate administration in acidosis, we have shown, using ³¹P-n.m.r., that pH_i in livers from DKA rats perfused at pH 6.9 is significantly higher than in similarly perfused livers from control animals (J. S. Beech, R. A. Iles & R. D. Cohen, unpublished work).

The apparent 'protection' of hepatic pH_i in the DKA group at low pH_e values requires some explanation, which can only be tentative at present. It is not related to better respiratory compensation, since there are no significant differences in $p\text{CO}_2$ among the groups. In the HCl group the fall in pH_i may simply be due to the

creation of an unfavourable gradient for the normal H^+ extrusion from the hepatocyte, e.g. by Na^+/H^+ exchange [25], a situation which exists in all three groups. In the NH_4Cl group the situation is more complex. The overall equation of urea synthesis from NH_3/NH_4^+ is usually written as:

i.e. there is a proton-generating reaction which might be considered to be wholly intracellular and therefore create an additional cell acidification over and above the gradient effect outlined above. However, it is likely that by far the greater proportion of nitrogen will enter the cell as NH_3 by non-ionic diffusion, leaving H^+ in the circulation. The reactions will therefore be:

The intracellular events (eqn. 3) are therefore neither acidifying or alkalinizing. There is therefore no reason why the relationship between pH_e and pH_i in the NH_4Cl series should be any different from in the HCl series, and this is what is found experimentally [Figs. 2(a) and 2(b)]. The situation in the DKA group is again different. There is substantial intracellular proton production during ketogenesis. Assuming that most of the lipid converted into ketone bodies originates peripherally, e.g. from adipose tissue, then eqn. (4) prevails initially:

Thus 3 protons are produced peripherally. The second step, conversion of palmitate⁻ into 3-hydroxybutyrate⁻ (3-OHB⁻), evolves a further 9 protons:

giving the overall reaction:

This reaction might be expected to produce a considerable lowering of hepatic pH_i in DKA. However, fat oxidation is a powerful stimulator of gluconeogenesis from C_3 compounds such as lactate and pyruvate, presumably because of the dependence of pyruvate carboxylase activity on acetyl-CoA [26], the supply of which is increased in DKA. There is substantial evidence (see above) that lactate enters the liver cell in both the un-ionized and ionized forms. The intracellular conversion of un-ionized lactic acid into glucose is proton-neutral. However, the intracellular conversion of the lactate ion into glucose consumes protons; and it has been demonstrated [27] that hepatic pH_i rises with the rate of lactate consumption. It is therefore possible that in the DKA series the proton-consuming consequences of the high rate of gluconeogenesis from lactate (stimulated by ketogenesis) more than counteract the proton-generating consequences of ketogenesis, and account for the 'protection' of hepatic pH_i in DKA. It is also possible that proton extrusion mechanisms are enhanced in DKA. Rosenthal *et al.* [28] have recently described a serum factor which enhances the Na^+/H^+ antiporter in hepatocytes from diabetic rats.

It should be pointed out that with the three models of

metabolic acidosis employed in this study it is very difficult to ensure that the time course of development of acidosis is similar in the three groups. In the NH_4Cl and HCl groups the acidosis is developed within 6 h. In the diabetic group it is likely that the major part of the acidosis developed over the 36–48 h period after the injection of streptozotocin, and it is thus not unlikely that the acidosis is relatively less acute than in the other groups. We cannot exclude the possibility that the differences observed in pH_i in the DKA group compared with the other groups are related to some unknown adaptive effect of prolonged acidosis on pH_i regulation in the liver in the DKA group, which might have occurred in the NH_4Cl and HCl groups if the acidosis had been more prolonged. Nevertheless, the central point remains that hepatic pH_i is scarcely affected in DKA.

The protection of hepatic pH_i in DKA may also explain the observation by Welbourn [29] that in DKA rats the liver remains a net remover of glutamine from the circulation, whereas in NH_4Cl acidosis the liver switches from the net removal of glutamine seen in animals of normal acid-base status to net output of glutamine. The phosphate-dependent glutaminase situated in the periportal cells of the hepatic lobule is strongly inhibited by acidosis [30], as is glutamine transport into the mitochondria [31]; such inhibition should not occur in DKA in which the pH_i fall is very limited.

In neither the diabetic nor the NH_4Cl groups was there any difference in ATP/ P_i ratio measured *in vivo* compared with controls. However, when $HClO_4$ liver extracts were analysed by ^{31}P -n.m.r., the glycerophosphoethanolamine content was found to be significantly lowered in the diabetic group compared with controls, though glycerophosphocholine content was unchanged. Cohen [32] found that ^{31}P -n.m.r. spectra of perfused livers taken from streptozotocin-diabetic rats had a raised P_i signal compared with non-diabetic controls, and no differences in glycerophosphoethanolamine were recorded. However, a chronic streptozotocin-diabetic model was used, compared with the acute model of the present study. Glycerophosphoethanolamine is produced from breakdown of phosphatidylethanolamine mediated by phospholipase, and the decrease in glycerophosphoethanolamine in DKA may reflect a change in balance between phosphatidylethanolamine synthesis and degradation.

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