Biochem. J. (1980) **192**, 191–202
Printed in Great Britain

Effects of fructose on the energy metabolism and acid–base status of the perfused starved-rat liver

A ^{31}phosphorus nuclear magnetic resonance study

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(*Received 20 March 1980/Accepted 27 May 1980*)

Fructose metabolism has been studied with ^{31}P n.m.r. in perfused livers from rats starved for 48h. The time course of changes in liver ATP, P_i and sugar phosphate (fructose 1-phosphate) concentrations, and intracellular pH were followed in each perfusion after infusion of fructose to give an initial concentration of either 5mM or 10mM. Rapid falls in the concentrations of ATP and P_i and intracellular pH occurred after infusion of fructose, reaching a minimum after 4–5min, which was lower in the 10mM group than in the 5mM group. These changes were accompanied by a rapid rise in fructose 1-phosphate, reaching a plateau also after 4–5min. At both concentrations of fructose, after the early falls, some recovery of ATP, P_i and intracellular pH occurred; this was complete for P_i and intracellular pH in the 5mM-fructose experiments (within 12–30min). Complete restoration of ATP to the pre-fructose value was not achieved in either the 5mM or 10mM groups. Measurements of the uptake of lactate by the liver indicated that the fall in intracellular pH was caused primarily by production of protons accompanying the formation of lactate from fructose with possibly a transient contribution generated during the rise in fructose 1-phosphate.

^{31}Phosphorus nuclear magnetic resonance (^{31}P n.m.r.) has been applied to the study of metabolism in perfused organs including heart and kidney (Jacobus *et al.*, 1977; Garlick *et al.*, 1977; Radda & Seeley, 1979; Sehr *et al.*, 1977) and more recently liver (Salhany *et al.*, 1979; McLaughlin *et al.*, 1979).

The method has the unique advantage of monitoring changes in phosphorus-containing tissue metabolites non-destructively. An additional advantage is that intracellular pH can be estimated since the frequency of the resonance of P_i is sensitive to pH in the physiological range (Moon & Richards, 1973; Hoult *et al.*, 1974; Burt *et al.*, 1976; Dawson *et al.*, 1977).

N.m.r. is inherently insensitive and it has usually been necessary to accumulate scans for 10min or more (Moon & Richards, 1973; Dawson *et al.*, 1977; Salhany *et al.*, 1979; McLaughlin *et al.*, 1979) when dealing with concentrations in the biological range (10^{-3}M). However, with improvements in superconducting magnets and radiofrequency coil design (Hoult & Richards, 1976), sufficient signal/noise may be obtained in 1–2min or less (Radda & Seeley, 1979) so that kinetic studies may be performed.

We have developed a system for studying the perfused rat liver by ^{31}P n.m.r. in a wide-bore n.m.r. spectrometer and have made observations on certain aspects of fructose metabolism. Fructose rapidly decreases the concentrations of P_i and ATP in the liver (Mäenpää *et al.*, 1968; Woods *et al.*, 1970; Sestoft, 1974; Bode *et al.*, 1974) and is eventually converted into both glucose and lactate. This has some clinical importance since fructose has been infused into patients as a source of intravenous carbohydrate and in this context has been implicated as a cause of lactic acidosis (Hers, 1970; Woods & Alberti, 1972; Sestoft, 1972; Cohen & Woods, 1976). However, to our knowledge no study of the acid–base changes involved has been made on the isolated liver.

Thus we have used ^{31}P n.m.r. to investigate the effects of fructose both on phosphorus-containing metabolites and also on hepatic cell pH.

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Materials

Animals

Male Wistar rats which had previously been fed on a normal diet were starved for 48h and weighed between 120 and 150g before perfusion.

Chemicals

All enzymes and NAD^+ were obtained from the Boehringer Corp. Ltd., Lewes, Sussex, U.K.; bovine serum albumin (fraction V) and L-(+)-lactic acid were obtained from Sigma Chemical Co., Poole, Dorset, U.K.

Methods

Perfusion

Isolated perfused liver preparations were set up as described before (Cohen *et al.*, 1973); however, both the perfusion circuit and the surgical technique had to be modified to comply with the constraints imposed by the n.m.r. spectrometer.

We designed a chamber for the liver made out of Perspex (Fig. 1) instead of the orthodox glass because of its ease of fabrication and durability. In our experience it has provided at least as good signals as those obtained with glass cells, perhaps because of its extremely homogeneous structure. The liver was held in a disc-shaped cavity within the chamber. The radiofrequency coil is also shown in Fig. 1; it provided excellent signal/noise ratios. The portal venous and the venous cannulae were constructed of poly(tetrafluoroethylene) and these and the two halves of the cell were sealed to give a water-tight chamber by means of the ring shown in Fig. 1.

A diagram of the perfusion circuit is shown in Fig. 2. The perfusion medium [Iles *et al.* (1979)] consisted of expired human erythrocytes resuspended in Krebs–Henseleit buffer (Krebs & Henseleit, 1932) containing 3% of bovine serum albumin to give a final packed cell volume of approx. 0.17. The pH of the perfusate was maintained at 7.35–7.45. Oxygenation was provided by O_2/CO_2 (95:5, v/v). Sodium L-(+)-lactate [prepared by neutralizing L-(+)-lactic acid] was added to give an initial concentration of approx. 2.5mM. An initial perfusate volume of 250ml was used for each experiment. The flow rate was maintained at 7–8ml (100g body wt.)$^{-1}$. The cannulation procedure was similar to that described by Cohen *et al.* (1973) except that both cannulae were attached to the sealing ring (Fig. 1) immediately before insertion and ligation into their respective blood

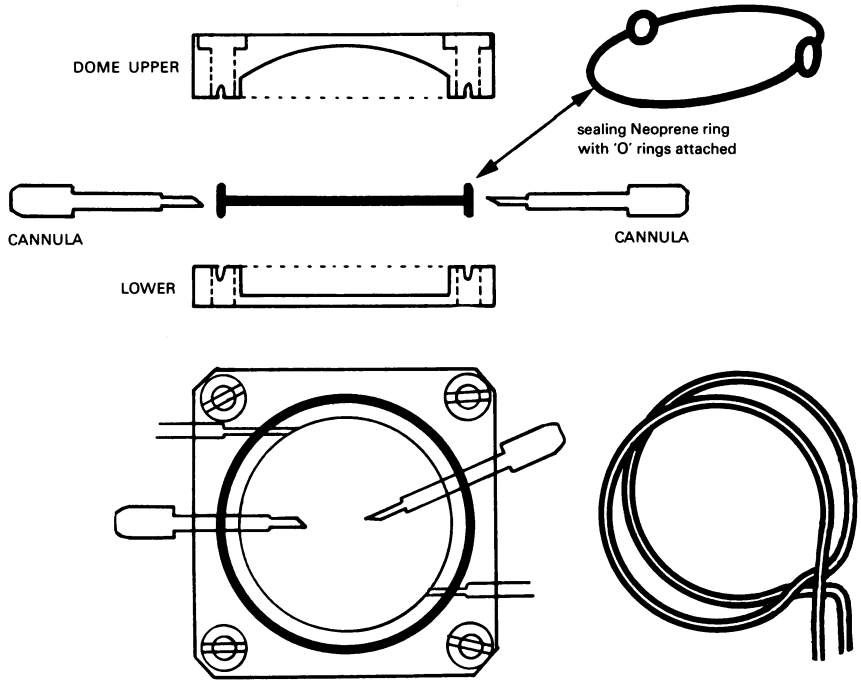

Fig. 1. *Radiofrequency coil and liver chamber*
The chamber, which contained the perfused liver, was mounted in the centre of the coil and located in the spectrometer probe as shown in Fig. 2.

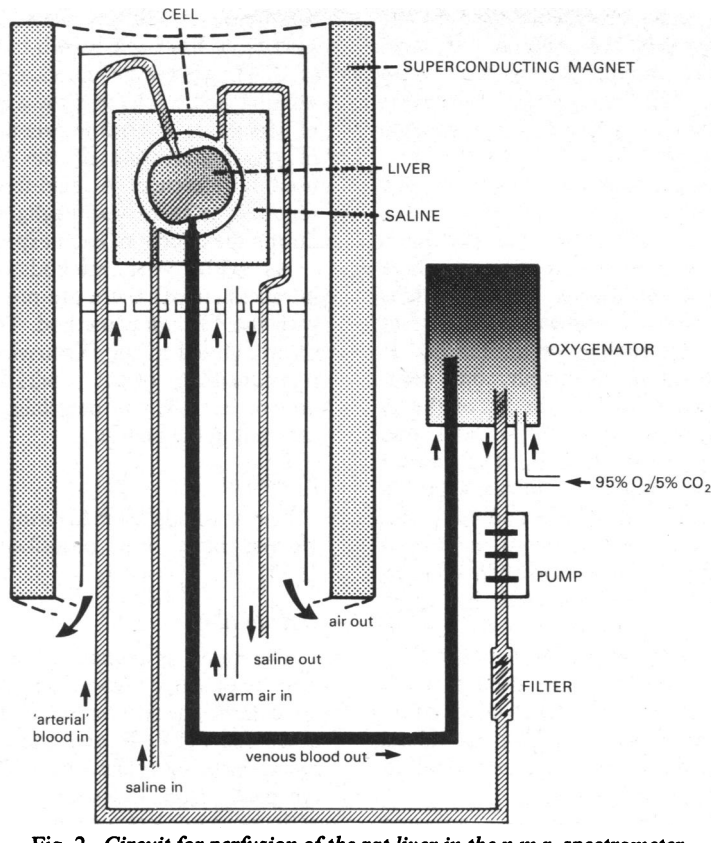

Fig. 2. *Circuit for perfusion of the rat liver in the n.m.r. spectrometer*

The perfusion medium consisted of Krebs buffer pH 7.4 containing erythrocytes (packed cell volume 17%) and albumin (3%) and initially 2–3 mM-lactate as substrate. The interior of the probe was maintained at 37°C and the oxygenator and filter were contained in a Perspex box maintained at 39°C. The flow rate was 7–8 ml min^{-1} (100 g body wt.)$^{-1}$. For further details see the text.

vessels. Perfusion was immediately converted from the non-recirculatory into the recirculatory mode and remained so for the duration of the experiment. The liver was removed from the rat carcass, first, by severing adhesions between the liver and the gut and, secondly, by excision of the heart and the majority of the diaphragm. The flow was then stopped, excision of the liver completed rapidly and, together with the cannulae and attached perfusion lines, the liver was transferred to one half of the Perspex chamber. The flow was then restored, after a total ischaemic period of less than 1 min, and the other half of the chamber secured by four nylon screws. The cell was then placed within the radio-frequency coil (Fig. 1) and filled with sodium chloride solution (150 mmol/l : saline) (Fig. 2); the probe was then inserted into the n.m.r. spectrometer magnet. Perfusions were continued for up to 3 h without apparent loss of liver function (as assessed by measurement of lactate uptake and glucose synthesis). The perfusions described in this paper were completed within 90 min.

After a 20–30 min equilibration period, several 2–4 min blocks of scans were accumulated and the ATP peaks of the spectra were compared by subtraction to ascertain whether the preparation was stable. In all experiments portal pressure remained below 20 cm water throughout perfusion. Samples of hepatic portal and venous perfusate were taken at various times for measurement of extracellular pH and lactate and glucose concentrations. After the equilibration period a volume of a solution of fructose was introduced rapidly into the reservoir to give an initial perfusate concentration of either 5 or 10 mM. Spectra were accumulated, usually in blocks of 1 or 2 min over the first 15 min of perfusion and then in blocks of 2 min or 4 min until the end of the experiment (50–60 min after fructose infusion).

N.m.r.

^{31}P n.m.r. spectra were recorded at 73.8 MHz on a spectrometer constructed in the Oxford laboratory (Hoult & Richards, 1975), equipped with a wide-

bore Oxford Instruments superconducting (4.3T) magnet and a Nicolet B-NC12 computer. In most experiments 80μs (60°) radiofrequency pulses were applied every 0.5s. The appropriate saturation factors were determined by applying radiofrequency pulses at intervals ranging from 0.05 to 4s. Spin-lattice relaxation time (T_1) measurements, performed by progressive saturation (Freeman & Hill, 1971), were made on two livers and on the perfusate. The results (not shown) indicated that the three peaks of ATP had a T_1 of approx. 100ms. This is much shorter than in other tissues, in agreement with the results of McLaughlin *et al.* (1979). P_i showed biphasic relaxation behaviour, one component of which corresponded to the T_1 of the P_i in the perfusion medium, approx. 3s, and was therefore assigned to extracellular P_i. The shorter component, which we assign to intracellular P_i had a T_1 of approx. 400ms. NAD and the compounds giving rise to the broad hump mentioned below have fairly long T_1 values and their concentrations may well be underestimated by rapid pulsing. We chose a pulse interval of 0.5s in contrast to a ^{31}P n.m.r. study on perfused mouse liver by McLaughlin *et al.* (1979). They used a much faster pulse rate, optimizing the signal of the ATP at the expense of the use of saturating conditions in which the peak areas are not proportional to the amounts of each compound present.

The magnetic field homogeneity was adjusted for each sample with the proton signal from the water within the sample. In other tissues the intracellular pH is often determined from the chemical shift difference between the ^{31}P resonances of phosphocreatine and P_i (Hoult *et al.*, 1974; Burt *et al.*, 1976; Dawson *et al.*, 1977) but there is no detectable phosphocreatine in the liver. We used the frequency of the water signal as a reference because it has been shown in the Oxford laboratory (J. J. H. Ackerman, D. G. Gadian & G. G. Wong, unpublished work) that the apparent pH of a variety of tissues is the same whether the ^{31}P resonance of phosphocreatine or the ^{1}H signal of water is used a reference. A field-frequency lock was not required as there was negligible magnetic field drift during the course of each experiment.

As with other methods for determination of intracellular pH there are possible errors associated with the ^{31}P n.m.r. method. The mean and standard error (quoted in the Discussion section) refer to the reproducibility of the results from liver to liver and do not exclude the possibility of systematic errors inherent in the method.

Chemical

The methods of measuring perfusate lactate and glucose have been described before (Iles *et al.*, 1977).

Calculations

The rates of lactate uptake and glucose output were calculated from the portal–hepatic venous concentration differences and the flow rate. Peak areas for ATP, P_i and sugar phosphate measurements were calculated by cutting out and weighing the peaks from the scan; this agreed satisfactorily with computer integration.

Results

Liver ^{31}P n.m.r. spectra

Fig. 3 shows a ^{31}P n.m.r. spectrum from a liver perfused with 2mM-lactate. The peak assignments are given in the legend. It is evident that there

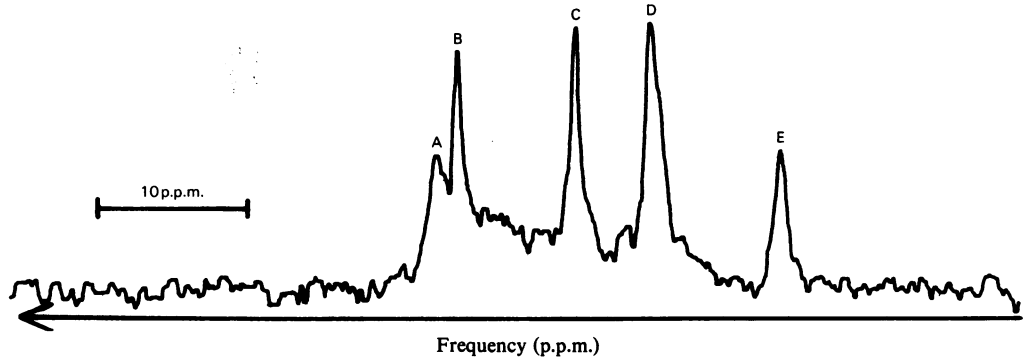

Fig. 3. *^{31}P n.m.r. spectrum of a perfused rat liver*

The liver was perfused with a medium containing 2mM-lactate. The spectrum represents the accumulation of 180 scans repeated at 0.5s intervals: peak A arises from sugar phosphates; peak B from P_i; peak C from the γ-phosphorus nucleus of ATP and β-phosphorus of ADP; peak D from the α-phosphorus of ATP and ADP, and the two phosphorus nuclei of NAD; peak E from the β-phosphorus of ATP.

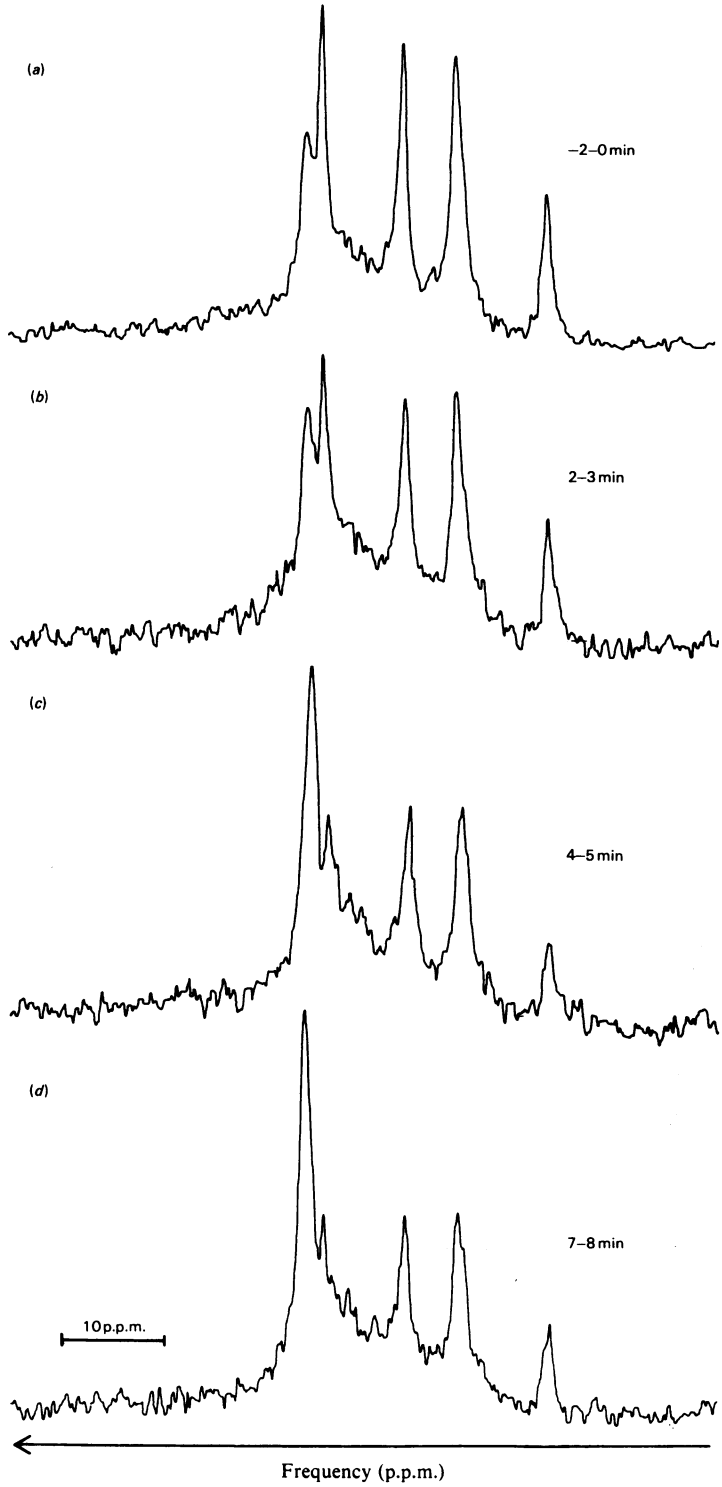

Fig. 4. *Series of ^{31}P n.m.r. spectra of a perfused rat liver, recorded as a function of time after addition of fructose to give a concentration of 10 mM in the perfusate*

The liver was perfused initially with a medium containing 2–3 mM-lactate before addition of fructose. Each spectrum represents the accumulation of 120 scans repeated at 0.5 s intervals and the times given are the mid-points of each accumulation.

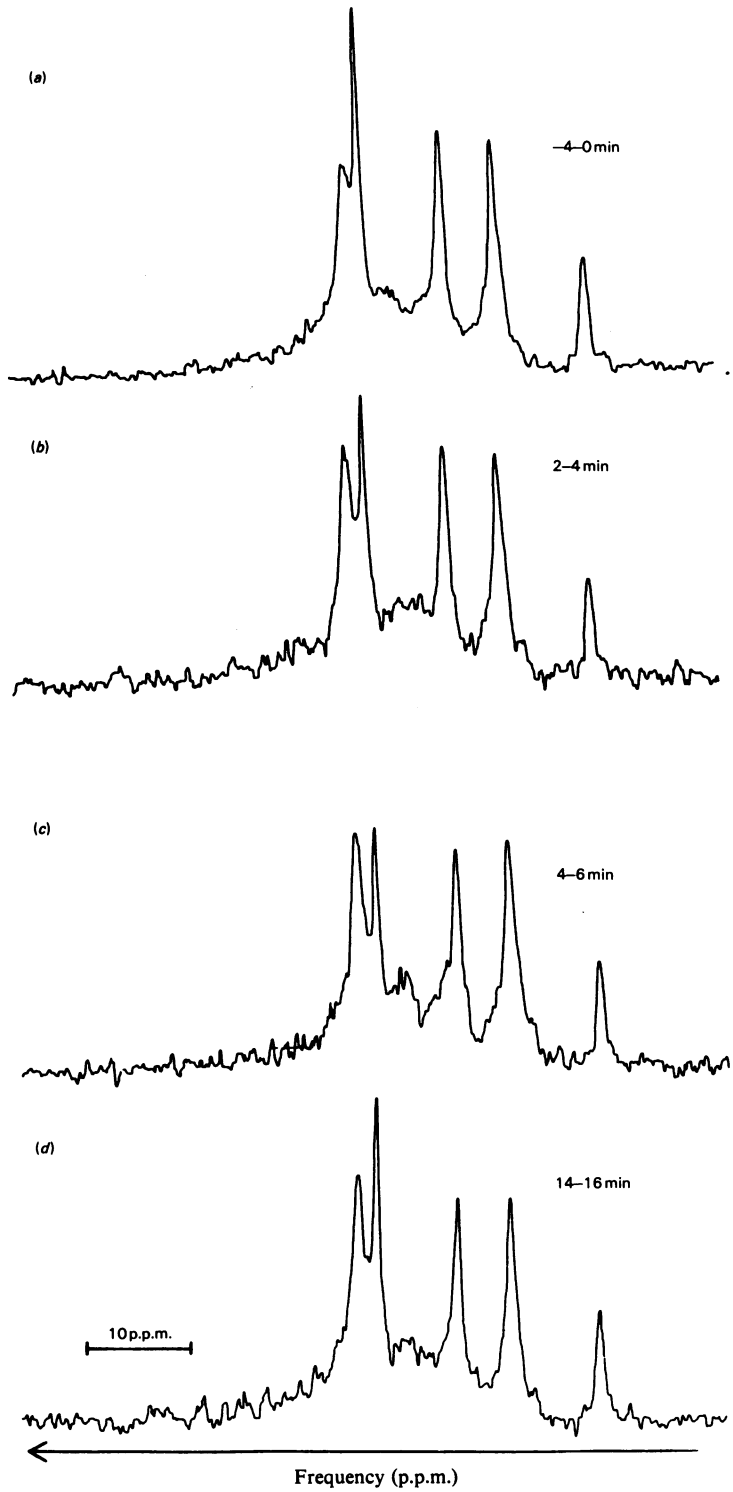

Fig. 5. *Series of ^{31}P n.m.r. spectra of a perfused rat liver as a function of time after addition of fructose to give a concentration of 5 mM in the perfusate*

The liver was perfused initially with a medium containing 2–3 mM-lactate before addition of fructose. Each spectrum represents the accumulation of 240 scans repeated at 0.5 s intervals and the times given are the mid-points of each accumulation. Peak assignments are as for Fig. 3.

is a broad hump underlying the sharper A–E peaks particularly noticeable in the P_i and sugar phosphate region. It is not known whether this represents phosphate-containing compounds bound to protein or present in some particular compartment in the cell. It is also found in heart (Radda *et al.*, 1979).

Figs. 4, 5 show sequences of liver spectra from two separate perfusion, before and after the addition of 10 and 5 mM-fructose to the perfusate. Spectrum (*a*) (Fig. 4) was taken immediately before infusion of 10 mM-fructose. Spectrum (*b*) was taken 2–3 min after the addition of fructose and it is apparent that a small decrease in ATP (E) and P_i (B) has occurred with a rise in sugar phosphate (A). Spectrum (*c*) was taken 4–5 min after the addition of fructose. ATP and P_i are now considerably decreased whereas the sugar phosphate peak is very high. After 7–8 min [spectrum (*d*)] the sugar phosphate peak had reached a maximum and ATP had partially recovered.

Fig. 5 shows a similar series of spectra from a 5 mM-fructose perfusion. Spectrum (*a*) was taken immediately before addition of fructose. Spectrum (*b*) 2–4 min later, shows a small decrease in ATP and P_i with a rise in sugar phosphate whereas (*c*), after 4–6 min, shows some recovery of the ATP and P_i peak with a fall in that of sugar phosphate. Spectrum (*d*), after 14–16 min, shows almost complete recovery of the ATP and P_i peaks.

Fig. 6 (*a,b*) show the time course in four perfusions of the changes in ATP and sugar phosphate respectively, expressed as a percentage of the control (pre-fructose) concentrations, after addition of 10 mM-fructose. ATP fell rapidly, within 3 min after fructose infusion, and reached a minimum of 25–45% of the control values within 5–6 min. This was followed by a partial recovery; an initial rapid phase of approx. 2 min duration was followed by a slow phase, which even after 40 min of perfusion did not result in full restoration of the ATP concentration (55–75% of control, results not shown). In three separate experiments livers were perfused for 60 min with 2–3 mM-lactate in the absence of fructose and no significant changes in phosphorus metabolites occurred.

P_i fell rapidly with a similar time course to ATP (results not shown), decreasing to 45–60% of the pre-fructose values after 3–4 min. However, in two experiments, after a fall to 60%, these were restored to their control values within 15 min whereas in the other two, after a greater fall (to 20–30% of control), a much slower recovery occurred approaching the pre-fructose value only after 40–50 min. The sugar phosphate concentration showed a rapid rise in all experiments followed by a plateau after 6–8 min.

Fig. 7 (*a,b*) show changes in ATP and sugar

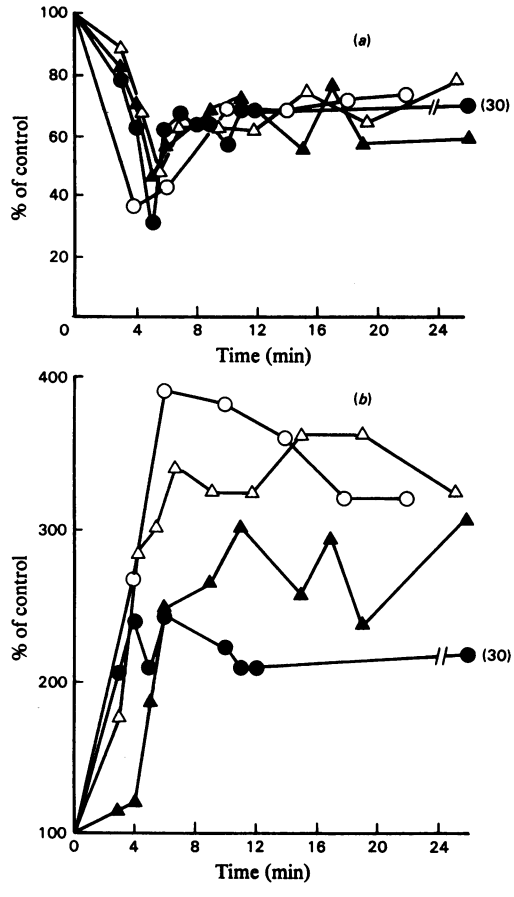

Fig. 6. *Time course of metabolite changes in perfused rat livers after addition of 10 mM-fructose*

Perfusion conditions were as for Fig. 3. The symbols (○, ●, △ and ▲) represent four perfusions. The concentrations of each metabolite are expressed as a percentage of their control (pre-fructose) values: (*a*) ATP; (*b*) sugar phosphate. Each point was obtained from the accumulation of 120–240 scans repeated at 0.5 s intervals and the times are the mid-points of each accumulation. Zero time is the point of addition of 10 mM-fructose to the perfusate.

phosphate in the three 5 mM-fructose perfusions. The initial fall in ATP in these experiments (to 60–70%) was less than that in the 10 mM-fructose perfusions. However, recovery was not complete (to 75–85%) even at this lower fructose concentration. P_i fell rapidly with a similar time course to the 10 mM-fructose perfusions (results not shown); however, the control values were restored in all three experiments within 15 min. Sugar phosphate concentrations rose rapidly but reached a lower plateau than the 10 mM-fructose perfusions.

Lactate and glucose metabolism

The results for lactate uptake and glucose output in the 10mM-fructose experiments are summarized in Table 1. Lactate uptake fell rapidly after addition of fructose and lactate output occurred within 8–13min. Lactate uptake was restored after 40min but did not achieve the pre-fructose rate. Glucose output increased rapidly (2–3-fold) to reach a plateau after 8–13min, which showed only a small decline after 35–40min.

Owing to technical difficulties it was not possible to synchronize sampling between the 5 and 10mM series. In the 5mM perfusions (Table 1) no lactate output was detected at 17–20min although the rate of lactate uptake was only a fifth of the pre-fructose value, rising to 55% of the latter at 35–40min. Glucose output had doubled at 17–20min

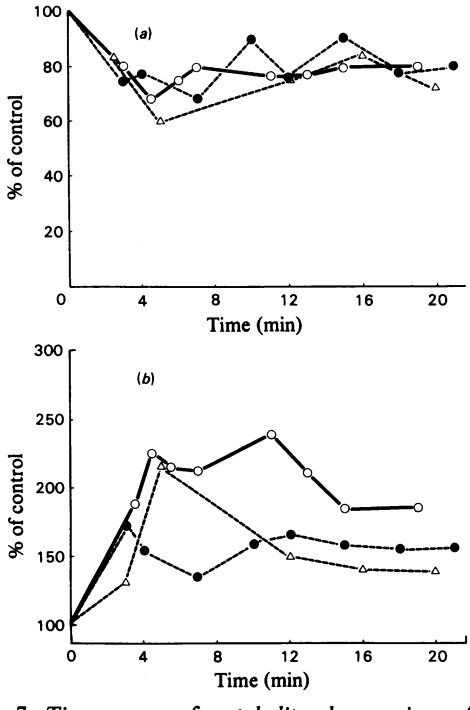

Fig. 7. *Time course of metabolite changes in perfused rat livers after addition of 5mM-fructose*

Perfusion conditions were as for Fig. 3. The symbols (○, ● and △) represent three perfusions. The concentrations of each metabolite are expressed as a percentage of their control (pre-fructose) value: (*a*) ATP; (*b*) sugar phosphate. Each point was obtained from the accumulation of 120–240 scans repeated at 0.5s intervals and the times are the mid-points of each accumulation. Zero time is the point of addition of 5mM-fructose to the perfusate.

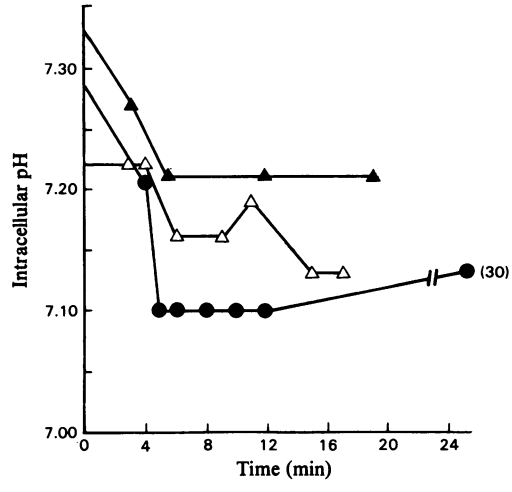

Fig. 8. *Time course of intracellular pH in perfused rat livers after addition of 10mM-fructose*

Perfusion conditions were as for Fig. 3. The symbols (●, △ and ▲) represent the same three perfusions as in Fig. 6. Each point was obtained from the accumulation of 120–240 scans repeated at 0.5s intervals and the times represent the mid-points of each accumulation. Zero time is the point of addition of 10mM-fructose to the perfusate.

Table 1. *Lactate uptake and glucose output in the perfused 48h starved-rat liver*

Livers from 48h starved rats were prepared with a medium containing initially 2–3mM-lactate. The times are measured from the point of addition of either 5 or 10mM-fructose to the reservoir. The results are means ± S.E.M. Significance of differences from the zero values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. n = number of observations.

	5mM-Fructose ($n = 3$)			10mM-Fructose ($n = 4$)		
Time (min)	0	17–20	35–40	0	8–13	35–40
Lactate uptake [μmol min^{-1} (100g body wt.)$^{-1}$]	6.08 ± 1.19	1.26 ± 0.18*	3.70 ± 1.12	7.63 ± 0.74	−1.77 ± 0.43***	1.06 ± 0.34***
Glucose output [μmol min^{-1} (100g body wt.)$^{-1}$]	2.49 ± 0.16	5.20 ± 0.43**	2.40 ± 0.25*	2.87 ± 0.36	7.05 ± 0.36***	6.25 ± 0.79*

Table 2. *Portal venous–hepatic venous pH difference in the perfused 48 h starved-rat liver*
Livers from 48h starved rats were perfused with a medium containing initially 2–3mM-lactate. The times are measured from the point of addition of either 5 or 10mM-fructose to the reservoir. The results are means ± S.E.M. Positive values indicate an output of protons by the liver. Significance of differences from the zero values: $^{**}P < 0.01$, $^{***}P < 0.001$. n = number of observations.

	5mM Fructose ($n = 3$)			10mM Fructose ($n = 4$)		
Time (min)	0	8–10	35–40	0	8–10	35–40
Portal venous–hepatic venous pH	−0.01 ± 0.001	0.04 ± 0.01	—	−0.04 ± 0.001	0.11 ± 0.01***	0.04 ± 0.01**

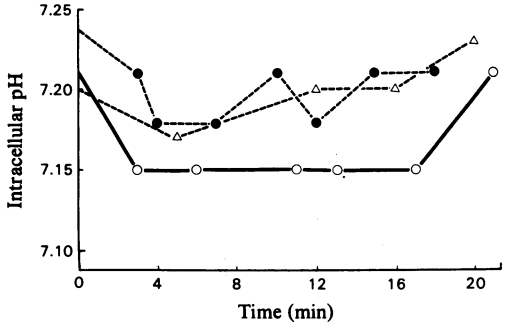

Fig. 9. *Time course of intracellular pH in perfused rat livers after addition of 5 mM-fructose*
Perfusion conditions were as for Fig. 3. The symbols (○, ● and △) represent the same three perfusions as in Fig. 7. Each point was obtained from the accumulation of 120–240 scans repeated at 0.5s intervals and the times represent the mid-points of each accumulation. Zero time is the point of addition of 5mM-fructose to the perfusate.

but was restored to the pre-fructose value at 35–40min.

pH changes

Addition of fructose resulted in a fall in intracellular pH in both 5mM and 10mM perfusions (Figs. 8 and 9). In the 10mM perfusions a rapid fall of 0.1–0.2pH unit occurred within 6min followed by a plateau until 30–40min when there was a slow rise to within 0.03–0.06 of the pre-fructose values. By contrast, in the 5mM experiments the fall in intracellular pH was smaller (0.03–0.06) and the pre-fructose values were restored within 20min.

Table 2 shows the changes in arteriovenous pH difference across the liver. In both 5mM and 10mM groups the pre-fructose difference was slightly negative but after 8–13min it became positive, i.e. a net output of acid from the liver was occurring. After 50min in the 10mM series this difference was considerably less but still positive.

Discussion

It is well established that fructose causes accumulation of fructose 1-phosphate in the liver (Kjerulf-Jensen, 1942; Määnpää *et al.*, 1968) and a rapid fall in ATP, both *in vivo* (Määnpää *et al.*, 1968) and *in vitro* (Woods *et al.*, 1970; Van den Berghe *et al.*, 1977). At the same time a fall in intracellular P_i occurs (Woods *et al.*, 1970; Sestoft, 1974; Van den Berghe *et al.*, 1977). The accumulation of fructose 1-phosphate was shown to be greater in fed than in starved rats (Sestoft, 1974).

The changes in intracellular phosphorus metabolites presented here are qualitatively similar to those recorded by previous workers. Fig. 6 illustrates one of the advantages of the n.m.r. technique in that changes in certain intracellular metabolites (in this case ATP) can be monitored repeatedly in a non-destructive manner. Although the ATP concentration is severely depressed by 10mM-fructose in the first 5–6min some recovery occurs quite rapidly and with 5 mM-fructose the changes are less severe, but in neither case is the original ATP concentration completely restored, presumably because of the irreversible degradation of a fraction of the adenylate pool to uric acid (Määnpää *et al.*, 1968, Van den Berghe *et al.*, 1977).

After 35–40min the ATP concentrations were 55–75% of the pre-fructose control values. The high activity of fructokinase and hence rapid phosphorylation of fructose presumably means that gluconeogenesis from lactate competes unsuccessfully for ATP. This is borne out by the observation that net lactate production occurs when fructose is metabolized both by livers of starved and fed rats (Woods *et al.*, 1970; Sestoft, 1974). In our present (10mM-fructose) study lactate uptake (Table 1) was negative during the period 8–13min after addition of fructose. Although this was not the period at which ATP concentrations were at their lowest (Fig. 6), they were still considerably depressed. We have no measurements of lactate uptake or glucose output in the 5mM series for the same time period (Table 1) but the 17–20min measurements indicate a considerable reduction of lactate uptake occurred.

In the 5mM perfusions, the glucose output at 35–40min was halved compared with the 17–20min period and the lactate uptake was restored to 61% of control. The fructose 1-phosphate concentration had declined somewhat at this time, which may have allowed a substantial amount of lactate to utilize ATP for conversion into glucose. In the 10mM series, although lactate uptake was restored at 35–40min, it was only 13% of control and no decline in the fructose 1-phosphate concentration had occurred.

The validity of the intracellular pH values obtained by n.m.r. depends on the ability to distinguish intra- from extra-cellular P_i. The liver chamber was designed to enable as large a proportion (at least 60%) as possible of its volume to be occupied by liver. However, the extracellular space (blood volume plus interstitial space) is high, between 30 and 35% of total tissue water (Iles *et al.*, 1979) and therefore it is likely that extracellular P_i will contribute to the observed peak. However, the relaxation time (T_1) for intracellular P_i is of the order of 400ms whereas the T_1 for blood is much longer (3s). Thus since we routinely pulse at 0.5s intervals the contribution of extracellular P_i is considerably less. In a control experiment (not shown) in which the chamber was filled with perfusion medium we found that the perfusate phosphate peak area was about 20% of that observed in the liver experiments. The contribution of extracellular P_i to the phosphate peak in liver spectra would be much less than this since the cell was then mainly filled with parenchymal tissue. Further evidence that we are measuring predominantly intracellular P_i comes from the chemical shift measurement, which gives a pH value 0.15–0.2 unit lower than either the portal venous or hepatic venous pH in the perfusate.

After fructose infusion it is likely that the initial fall in P_i is due to loss of intracellular P_i; the extracellular pool may therefore comprise a greater fraction of the residual P_i. This would result in an underestimate of the fall in both intracellular P_i and pH; however, the chemical shift indicates a pH which is still 0.15 unit lower than the extracellular pH.

It would perhaps be feasible to use a phosphate-free perfusate; however, since it has been shown by Sestoft (1974) that a small leakage of P_i from the perfused liver occurs even in the presence of a P_i containing medium, there may be a deleterious effect on the organ.

The fall in P_i after fructose infusion was very rapid (3–4min) and in the three 5mM-fructose perfusions and two of the 10mM-fructose perfusions complete recovery occurred after 14min. Sestoft (1974), observed a rapid uptake of P_i from the medium after fructose addition and it is presumably this process and the balance between the rates of synthesis and degradation of fructose 1-phosphate that determine the intracellular concentration. The P_i concentration often tended to overshoot by 10–20% of the original value 15–25min after fructose infusion (see Määnpää *et al.*, 1968).

Many compounds containing a phosphorylated sugar residue contribute to the sugar phosphate peak (Fig. 3). After addition of fructose the increase in peak area (Figs. 4 and 5) is caused mainly by the substantial increase in fructose 1-phosphate (Woods *et al.*, 1970). It is possible, however, that about one-third of this area is contributed by an increase in IMP, which was shown by Woods *et al.* to occur in perfused livers of fed rats in the presence of 10mM-fructose.

The fall in intracellular pH after fructose addition is rapid and follows a similar time course to the fall in ATP. Part of the fall may be due to a release of protons accompanying the phosphorylation of fructose as shown in eqn. (1). (The overall stoicheiometry is not exactly 1 proton/mol of fructose; we have used the predominant ionized forms at the prevailing pH.)

$$\text{Fructose} + MgATP^{2-} \rightarrow \text{fructose 1-}P^{2-} + MgADP^{1-} + H^+ \quad (1)$$

This proton release is transient, however, since it is limited by the magnitude of the initial increase in fructose 1-phosphate concentration and fall in ATP concentration. Further metabolism of fructose 1-phosphate to glucose and reconversion of ADP into ATP, to maintain the new steady-state concentrations, will result in a net balance of zero protons released. However, at the same time it is apparent that a change from lactate uptake, which by eqn. (2) results in a net uptake of protons, to lactate output has occurred (eqn. 3).

$$2\ \text{Lactate}^- + 2H^+ \rightarrow \text{glucose} \quad (2)$$

$$\text{Fructose} \rightarrow 2H^+ + 2\ \text{lactate}^- \quad (3)$$

Continued production of protons could thus only occur by this mechanism, which presumably accounts for the continued suppression of intracellular pH (Fig. 8). In the 10mM series, after 40min, there is an almost complete return of the intracellular pH to pre-fructose values and this coincides with the restoration of lactate uptake after net output. In the 5mM series the recovery of pH was complete and within 20min of fructose addition lactate uptake was re-established.

Previous determinations of intracellular pH in the perfused rat liver with the weak acid indicator 5,5′-dimethyloxazolidine-2,4′-dione have given a mean value of about 7.24 when the perfusate pH was 7.4 (Cohen *et al.*, 1971; Lloyd *et al.*, 1973; Cohen & Iles, 1975). With a similar substrate con-

centration (2mM-lactate) the mean pre-fructose value obtained by the n.m.r. method in the present study was 7.25 ± 0.02 (S.E.M., $n = 7$). The agreement is good, although in the experiments of Cohen *et al.* (1971) and Lloyd *et al.* (1973) the pCO_2 was monitored throughout the perfusions and kept at a mean value of about 5.3kPa. It was not possible to measure PCO_2 in the present study and it was assumed to be approximately 4.7kPa.

Two publications on ^{31}P n.m.r., in perfused rat (Salhany *et al.*, 1979) and mouse liver (McLaughlin *et al.*, 1979), have appeared: Salhany *et al.*, estimated a pH of 7.4 from the chemical shift of P_i, which they assigned to extracellular P_i since on poisoning the liver with cyanide a new peak appeared in the P_i region which gave a pH of 7.05. McLaughlin *et al.* also observed a peak at a pH of 7.4 and concluded that this was due to intracellular P_i.

The n.m.r. method of measuring cell pH has considerable advantages over the weak acid–base indicator method. First, it is non-destructive whereas the latter method requires biopsies and makes repeated measurements exceedingly difficult. Secondly, the weak acid–base method requires the presence of an equilibrium between intra- and extra-cellular concentrations of the indicator so that rapid disturbances of acid–base status cannot be monitored. This problem does not arise with the n.m.r. method, the only limitation being that the time required for accumulating an adequate number of scans should not be too long compared with the rate of change of intracellular pH. If the latter is very rapid, changes may be obscured because n.m.r. gives an integrated value over the period of scanning.

It has been demonstrated by Woods & Alberti (1972) that fructose infusion can give rise to systemic acidosis. In a normally functioning, well-oxygenated liver, it seems likely that the acidosis will be minor. Severe acidosis may develop only when the organ is inadequately oxygenated, e.g. if it is poorly perfused, and under these conditions a greater fraction of the fructose may be converted into lactic acid rather than glucose (Woods & Krebs, 1971). In some preliminary experiments we have observed falls of 0.5–0.8 in hepatic pH in livers perfused with 10mM-fructose when erythrocytes are omitted from the perfusion medium, with oxygen delivery 30% of that under our normal perfusion conditions.

A preliminary report of this work has been presented before (Iles *et al.*, 1980).

We are grateful to Mr. C. Browning and Mr. L. Blow for their help in designing the liver chamber and building the probe. We thank Professor R. D. Cohen for his encouragement and helpful discussion and Miss E. A. Wilkie for her skilful technical assistance. We thank the Science Research Council for support and a Studentship award to A. N. S.

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