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A Study of Galactose Intolerance in Human and Rat Liver *In Vivo* by ^{31}P Magnetic Resonance Spectroscopy

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ABSTRACT. An oral load of 20 mg/kg galactose produces significant changes in the ^{31}P magnetic resonance spectrum of the liver of a galactosemic patient. The peak at 5.2 ppm (which includes inorganic phosphate and galactose-1-phosphate) increased on two occasions to about twice its original size 60 min after galactose administration. An oral load of 10 mg/kg galactose given to a second patient produced no discernible changes at 30 min. We have also used an animal model of galactose intolerance, in which galactose metabolism in rats was blocked by the acute administration of ethanol. Studies *in vivo* and *in vitro* showed that the increase in the peak at 5.2 ppm was largely due to galactose-1-phosphate. We have shown in this preliminary study that small amounts of galactose can produce significant elevation of hepatic galactose-1-phosphate, which can be detected by ^{31}P magnetic resonance spectroscopy. (*Pediatr Res* 32: 39–44, 1992)

Abbreviations

MRS, magnetic resonance spectroscopy
MR, magnetic resonance
P_i, inorganic phosphate
UDP, uridine diphosphate

Galactosemia is an autosomal recessive disorder caused by a deficiency of the enzyme UDPglucose:α-D-galactose-1-phosphate uridylyltransferase (EC 2.7.7.12) (Fig. 1) (1). In infancy, ingestion of galactose by patients with galactosemia causes liver disease, cataract, and failure to thrive, but with strict exclusion of galactose there is rapid improvement with resolution of the liver disease and normal growth. The diagnosis of galactosemia is normally confirmed by measuring the enzyme activity in red blood cells, but the major site of galactose metabolism is the liver (2). The liver damage is thought to be caused by accumulation of metabolites of galactose, although the exact mechanism is unclear (1). We have used MRS of the liver to determine the fate of galactose noninvasively in this disorder. We have previously shown in another hereditary disorder, fructose intolerance, that it is possible to detect noninvasively the effects of a small amount of fructose on the liver by means of ^{31}P MRS (3). In this study, we show that small amounts of oral galactose produce significant changes in ^{31}P MR spectra of the liver. In an animal model in which galactose metabolism is partially blocked, we show that the changes seen in the patient are probably due to the accumulation of galactose-1-phosphate. This model uses acute administration of ethanol, which inhibits UDPglucose-4-epimerase (EC 5.1.3.2) (Fig. 1) by increasing the NADH/NAD^+ ratio in the liver. Although this is not the enzyme that is deficient in galactosemia, its inhibition blocks the usual pathway of galactose metabolism, leading to an accumulation of galactose-1-phosphate and UDPgalactose (4, 5).

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MATERIALS AND METHODS

Patients. Two patients with galactosemia were studied (Table 1). The patients or their guardians gave informed consent to the study, which was approved by the local ethics committee. Both were on a strict galactose-free diet and were studied after an overnight fast. The two patients were well controlled, with red blood cell galactose-1-phosphate concentrations usually within the normal range (<0.57 μmol/g Hb).

MRS. ^{31}P MR spectra were obtained with a 1.9-tesla, 60-cm bore superconducting magnet operating at 32.7 MHz for phosphorus. The patient lay prone with her liver positioned over a 8-cm diameter surface coil. The spectra were localized with a field-profiling technique (6). This method (now largely superseded by more accurate localization strategies) sometimes gives rise to spectra with a contribution from overlying skeletal muscle (as evidenced by the phosphocreatine peak in Fig. 2). From the known phosphocreatine/ATP ratio in muscle under these acquisition conditions, we estimate that less than 10% of the nucleoside triphosphate peaks in the spectrum can be from muscle, and much smaller proportions of the phosphomonoester, P_i, and phosphodiester peaks. The interpulse delay was 1 s, with a pulse angle of 90° in the liver. Patient 1 was given 20 mg/kg galactose orally and patient 2 was given 10 mg/kg. Spectra were collected from patient 1 at 5-min intervals before and for up to 60 min after the administration of galactose. In a second study on this patient, brain and liver spectra were collected. After the administration of 20 mg/kg galactose, brain spectra were collected for 40 min, followed by a final liver spectrum at 60 min. In the study on patient 2, spectra were collected before and at 30 min after the galactose dose. Results are expressed as a percentage of the mean pregalactose peak areas.

Animal study. In the animal study, male Wistar rats (200–300 g) were fasted for 24 h, with free access to water. The care of the animals at all times conformed to the Home Office guidelines for the care and use of laboratory animals. The rats were anesthetized with nitrous oxide and halothane and were nephrectomized, and the femoral vein was cannulated. Ethanol (100–150 mg/100 g body weight) was infused via the cannula, followed after 15 min by galactose (45–100 mg/100 g). In a control group, saline replaced the ethanol. ^{31}P MR spectra were obtained at 1.9 tesla at 15-min intervals throughout the experiment by means of a surface coil inserted between the lobes of the surgically exposed liver. Twenty to 30 min after infusion of galactose, the livers were removed and freeze-clamped.

The frozen livers were ground into a powder under liquid nitrogen and extracted with 6% perchloric acid (about 3 mL/g of liver). The extracts were centrifuged, neutralized with KOH, freeze-dried, and dissolved in EDTA (15 mM) at pH 8.5. The solutions were studied by ^{31}P MRS at 121 MHz. Spectra were obtained with a 45° pulse and an interpulse delay of 5 s, with broad-band proton decoupling (with a WALTZ sequence) during acquisition. Compounds were identified by their chemical shifts in comparison with published values, by their pH titration behavior, and by adding authentic samples of suspected compounds. Peak areas were quantified with the spectrometer's integration routine, and metabolite levels were expressed as percentages of total acid-extractable phosphorus. Chemical shifts are relative to phosphocreatine at 0 ppm, with glycerophosphocholine at 2.9 ppm acting as an internal chemical shift standard.

RESULTS

Patients. Patient 1 showed an initially normal ^{31}P MR liver spectrum (all metabolite ratios being within 2 SD of the control mean), whereas patient 2 had a P_i/ATP ratio higher than the normal range (Table 1). Control data from subjects of this age group are not available, but our studies of children aged 5 and 6 y with other conditions showed liver spectra indistinguishable from those of adults. Spectra from the livers of neonates are different from the adult spectra in that the phosphomonoester signals are relatively larger (7).

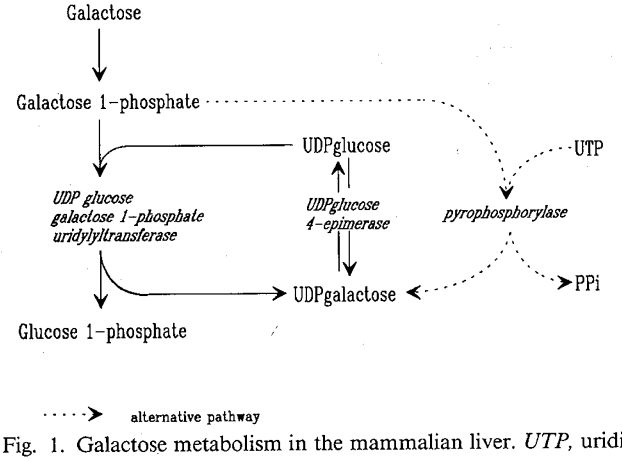

Fig. 1. Galactose metabolism in the mammalian liver. *UTP,* uridine triphosphate; *PPi,* pyrophosphate.

The effect of administration of 20 mg/kg galactose to patient 1 on her ^{31}P MR liver spectrum is shown in Figure 2. There was an increase in the intensity of the peak at 5.2 ppm, which includes P_i and galactose-1-phosphate, and the time course of the changes is shown in Figure 3. The area of the peak at 5.2 ppm reached 150% of its initial value after 30 min, rising to 180% at the end of the study. The areas of the ATP peaks were unchanged over this time. In the second study on the same patient, normal brain and liver ^{31}P MR spectra were again obtained. After the administration of 20 mg/kg galactose, the brain spectra were unchanged over 40 min. A liver spectrum obtained 60 min after the galactose dose showed an increase in the peak at 5.2 ppm to 223% of its original area. Patient 2, given 10 mg/kg galactose, showed no significant changes in a spectrum obtained 30 min after the dose. A smaller dose was chosen for this patient in view of her age (7 y) and the large changes seen in patient 1.

Animal study. The animal study *in vivo* showed similar but less marked changes in animals treated with ethanol and galactose compared with the patient (Fig. 4). The peak at 5.2 ppm (P_i and galactose-1-phosphate) increased by 20 ± 3% relative to before treatment, but the ATP β-P declined over the same time course (Table 2). An increase in the phosphomonoester region was seen in some animals after ethanol administration, before infusion of galactose. The saline and galactose-treated animals showed no significant changes.

The spectra of extracts (Fig. 5 and Table 3) show that galactose-1-phosphate (in the P_i region) and UDPgalactose (in the ATP α-P region) were both increased in the ethanol and galactose-treated animals to about 2.5 times the amounts found in the saline and galactose-treated animals, whereas a slight decrease in ATP and increase in P_i were seen in both groups, compared with untreated controls. Neither galactose-1-phosphate nor UDPgalactose was detectable in untreated (fasted) rat liver extracts. The spectra of extracts show that the ratio of UDPgalactose to galactose-1-phosphate was about 1.5:1, both in the ethanol and galactose-treated group and in the saline and galactose-treated animals, suggesting that inhibition of UDPglucose-4-epimerase by ethanol does not alter the product-to-substrate ratio of UDPglucose:galactose-1-phosphate uridylyltransferase, which is the previous enzyme in the pathway. The epimerase reaction is considered to be near equilibrium in the liver. *In vitro* studies of UDPglucose-4-epimerase have shown the ratio of UDPglucose to UDPgalactose to be about 3:1 at equilibrium (4), but this ratio is shifted toward UDPgalactose when the epimerase is inhibited. We found that the UDPglucose:UDPgalactose ratio altered from about 3:1 in the saline-treated rat liver to about 1:5 in the ethanol and galactose-treated animals (Fig. 5), thus our results are consistent with inhibition of the epimerase.

This is further supported by the ratios of *sn*-3-glycerol phosphate, phosphoenol pyruvate and 3-phosphoglycerate measured in the liver extracts (Fig. 6 and Table 4). Ethanol inhibits UDPglucose-4-epimerase by altering NADH/NAD^+ ratios, NADH being a potent inhibitor (5, 8). A higher cytosolic NADH/NAD^+

Table 1. *Details of patients*

	Sex	Age (y)	Red blood cell uridylyltransferase (μmol substrate converted/h/g Hb)	Red blood cell galactose-1-phosphate (μmol/g Hb)	Hepatic P_i/ATP (MRS ratio)
Patient 1					
1st MRS study	F	20	0.2	0.12	0.87
2nd MRS study				0.60	0.75
Patient 2	F	7	0	0.69	1.06
Normal levels			>18	<0.57	
Controls (for MRS study)		20–45			0.64 (±0.3)*

* Mean ± 2 SD; n = 23.

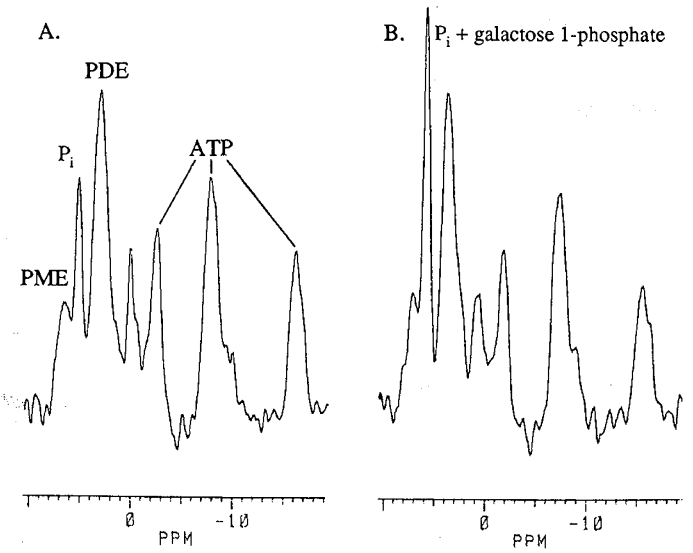

Fig. 2. ^{31}P MR spectra *in vivo* of the liver of a galactosemic patient before (*A*) and after (*B*) administration of 20 mg/kg galactose. The peak at 0 ppm is from phosphocreatine in overlying muscle. The peaks labeled ATP contain contributions from other nucleoside triphosphates and diphosphates as can be seen in the extract spectra in Figure 5. The signal from the β-phosphate of ATP at −16 ppm contains about 10% guanosine 5′-triphosphate. *PME*, phosphomonoester, *PDE*, phosphodiester.

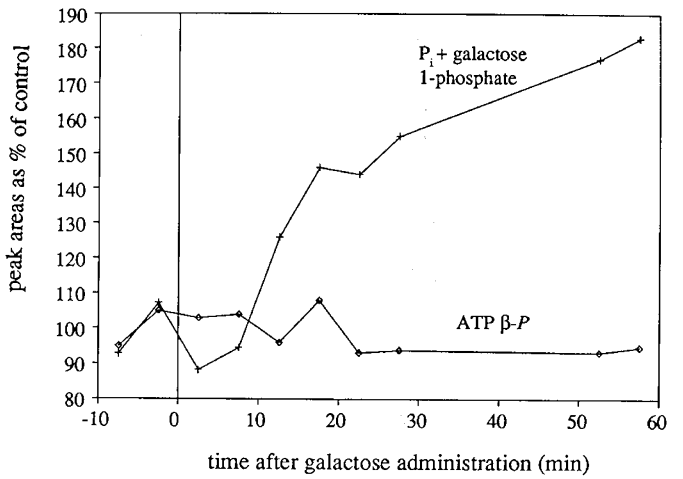

Fig. 3. Relative peak areas in the patient's liver spectrum after the administration of galactose (mean initial values set to 100%).

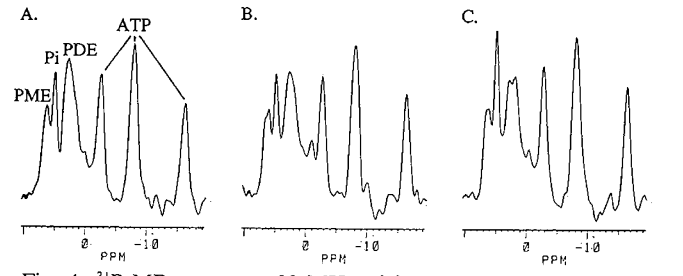

Fig. 4. ^{31}P MR spectra at 32 MHz of fasted rat liver *in vivo* before treatment (*A*), and after sequential infusions of ethanol (*B*), and galactose (*C*). All spectra are from a sequence of experiments in a single animal. Details of MRS are in the text. The peaks labeled ATP contain contributions from other nucleoside triphosphates and diphosphates, as can be seen in the extract spectra in Figure 5. The signal from the β-phosphate of ATP at −16 ppm contains about 10% guanosine triphosphate. *PME*, phosphomonoester; *Pi*, inorganic phosphate; *PDE*, phosphodiester.

Table 2. *Animal study: in vivo hepatic metabolite levels as % of pretreatment values [mean (SEM)]**

Treatment	*n*	PME	P$_i$	ATP β-P
Ethanol + galactose	7	114	119†	87†
		(6)	(3)	(4)
Saline + galactose	4	103	97	98
		(1)	(3)	(1)

* PME, phosphomonoester; ATP β-P, ATP β-phosphate.
† $p < 0.01$ compared to before treatment (paired *t* test).

ratio would be expected to give increased levels of *sn*-3-glycerol phosphate and decreased levels of phosphoenol pyruvate and 3-phosphoglycerate by the effect of this ratio on the equilibria of glycerol phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase. The results in Table 4 confirm that the ethanol-treated animals indeed had a markedly raised 3-glycerol phosphate/3-phosphoglycerate ratio, and therefore the increased NADH/NAD$^+$ ratio may be inferred. The increase in *sn*-3-glycerol phosphate may explain the change in the phosphomonoester region of the spectra *in vivo* after the administration of ethanol.

DISCUSSION

This study shows that in galactosemia, a small oral galactose load can produce large changes in the ^{31}P MR spectra of the liver. The metabolite ratios in the unstressed liver were normal in one of the patients, whereas the second patient had a P$_i$/ATP ratio just above the normal range, the other metabolite ratios being normal. There seemed to be little correlation between resting red cell galactose-1-phosphate levels and liver P$_i$/ATP ratios (Table 1).

An oral galactose load of 20 mg/kg given to patient 1 was followed by a rise in the region of the ^{31}P MR spectrum that includes P$_i$ and galactose-1-phosphate, following the time course shown in Figure 3. It is likely that most of the increase in this peak was due to accumulation of galactose-1-phosphate, which in galactosemic patients is not converted to glucose-1-phosphate. In the MR spectrum *in vivo*, however, the galactose-1-phosphate and P$_i$ signals are too close together to be resolved. There may be a contribution from P$_i$ to this signal; Isselbacher (9) has proposed an alternative pathway (Fig. 1) for the conversion of galactose-1-phosphate to UDPgalactose, with the release of pyrophosphate, further hydrolyzed to P$_i$. The activity of the pyrophosphorylase (galactose-1-phosphate uridylyltransferase EC 2.7.7.10) *in vivo*, however, is thought to be quite low, so the release of P$_i$ from this reaction is unlikely to be a major contributor to the increase seen in the peak *in vivo*. In a second study, we monitored changes in the patient's brain over the same time course. Spectra were collected from her brain and liver before the administration of 20 mg/kg galactose, then brain spectra were collected for 40 min after the dose. No changes were observed during this time. The patient was then repositioned, and a liver spectrum was obtained (60 min after the galactose dose). The peak at 5.2 ppm had increased to a similar extent as it had at 60 min in the previous study.

The large change seen in the first patient's liver spectrum after 20 mg/kg galactose suggested that this dose could be deleterious in view of the possible toxicity of galactose-1-phosphate, particularly in younger patients. We were also interested in determining the sensitivity of the method at lower galactose loads. We therefore administered only 10 mg/kg galactose orally to patient 2. No changes were seen in her ^{31}P MR spectrum at 30 min after the dose. The reasons for this are unclear, but it was not possible to follow the complete time course in this patient, and so a transient change may have been missed.

In fructosemic patients, the chemical shifts of fructose-1-phosphate and P$_i$ are sufficiently different for them to be distinguished and separately quantified *in vivo* (3). Because this was not pos-

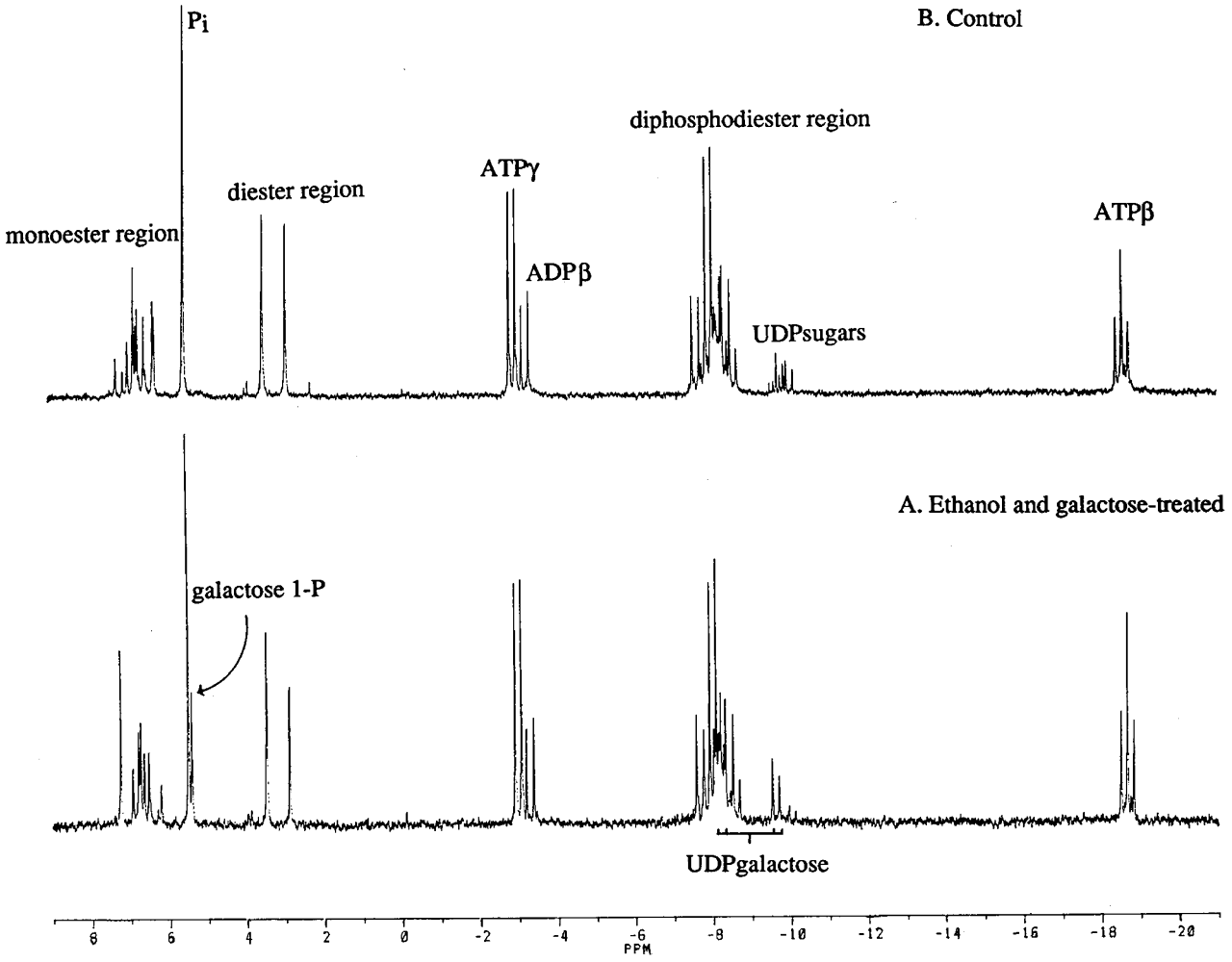

Fig. 5. ^{31}P MR spectra of the perchloric acid extracts of fasted rat liver at 121 MHz. *A*, Ethanol and galactose-treated; *B*, control. Sweep width 6000 Hz, transformed with gaussian window (time constant 1 s).

Table 3. *Animal study: in vitro levels as % of total phosphorus-containing metabolites [mean (SEM)]**

Treatment	*n*	P_i	Galactose-1-P	ATP	UDPgalactose
None	11	8.5 (0.6)	ND	10.9 (0.3)	ND
Saline + galactose	4	9.2 (1.4)	0.9 (0.1)	9.3 (0.8)	1.9 (0.1)
Ethanol + galactose	11	10.2 (0.5)	2.1† (0.2)	8.6 (0.5)	3.1† (0.2)

* Galactose-1-P, galactose-1-phosphate; ND, not detectable.

† $p < 0.01$ compared to saline + galactose administration and compared to no treatment.

sible in the galactosemic patients, we studied an animal model to elucidate the changes in P_i and galactose-1-phosphate. Rats were rendered galactose intolerant by inhibiting the epimerase with ethanol. High-resolution spectra of liver extracts revealed separate peaks for galactose-1-phosphate and P_i (Figs. 5 and 6). The increase in the P_i region was found to be due almost entirely to galactose-1-phosphate, with only a small contribution from an increase in P_i (Table 3). The small decrease in ATP compared with controls was not statistically significant, although there was a small but significant decrease in ATP in the spectra *in vivo* after ethanol and galactose treatment. The relative galactose load was up to 50 times greater than that in the human study.

If the changes after galactose treatment in the rat are similar to those in the human liver, then it is possible to explain the relative absence of hyperuricemia and clinical gout in patients with galactose intolerance compared to patients with either fructose intolerance or glycogen storage disease. In fructose intolerance, fructose leads to significant depletion of ATP (partly due to trapping as fructose-1-phosphate) and large swings in P_i. Depletion of ATP activates the adenylate kinase reaction (2 ADP → ATP + AMP), increasing AMP in an attempt to reestablish ATP levels. Swings in P_i concentration are known to activate rate-limiting enzymes in uric acid production (phosphoribosylpyrophosphate synthetase is activated by high P_i (10), whereas AMP deaminase is activated by low P_i (11), both of which can lead to increased purine production and and therefore to increased serum urate). Patients with glycogen storage disease type I show depletion of ATP and P_i during fasting and an increase in P_i after glucose (10, 12). Thus, in both fructosemia and type 1A glycogen storage disease, the precursor for uric acid production (*i.e.*, AMP) is present and there is an increase in enzyme activity, so an increase in serum urate can be expected. In galactosemia, the data from rats suggest that there is minimal increase in P_i, and the human data shows no decrease in ATP, so that the metabolite sequelae are not the same as in fructose intolerance or glycogen storage disease. The reasons for the differences between the ATP levels in these conditions are not clear. It is difficult to understand why the hepatocytes cannot rephosphorylate ADP in patients with fructosemia or glycogen storage disease if they can do so in patients with galactosemia.

In conclusion, oral galactose produces large changes in the ^{31}P MR spectra of the liver that are likely to be due to accumulation of galactose-1-phosphate. These changes were not seen in the brain over the same time course, confirming the importance of

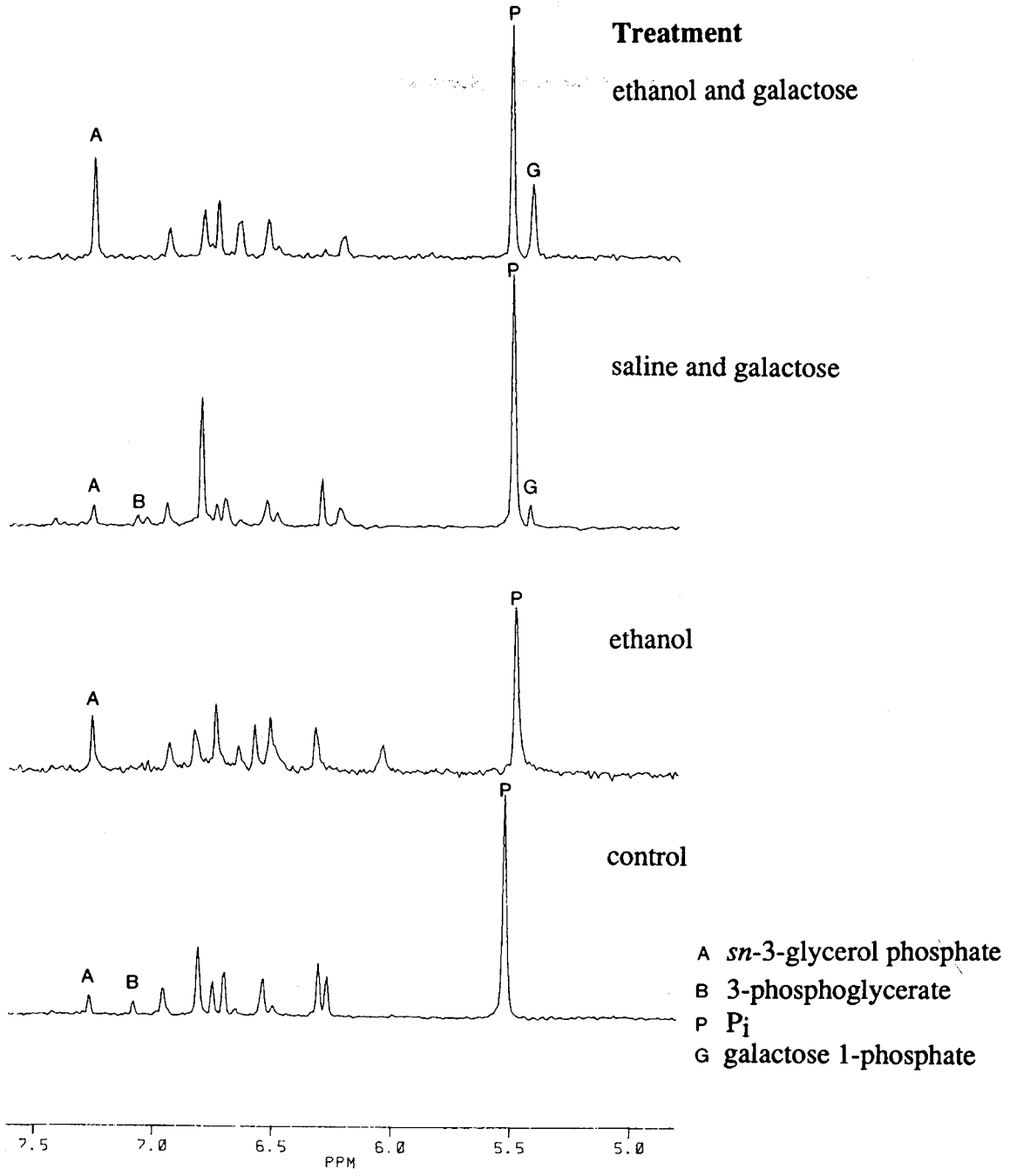

Fig. 6. Expansion of phosphomonoester and P_i regions of extract spectra. Slight differences in the positions of peaks are due to pH variations.

Table 4. *Animal study: in vitro levels of intermediary phosphorus-containing metabolites, as % of total phosphorus-containing metabolites [mean (SEM)]*

Treatment	*n*	*sn*-3-Glycerol phosphate	3-Phospho glycerate	Phosphoenol pyruvate
Fasted control	11	1.1 (0.3)	0.5 (0.1)	0.2 (0.1)
Saline + galactose	4	1.6 (0.3)	0.5 (0.1)	0.2 (0.1)
Ethanol + galactose	11	3.4 (0.3)	<0.1	<0.1

the liver as the initial site of galactose metabolism. Thus, this preliminary study suggests that ^{31}P MR spectroscopy can be used to monitor time-dependent changes in the liver of galactosemic patients, despite the fact that the changes were found in only one of two patients. The reason for this was either the smaller dose given to the second patient or the fact that the liver spectrum was only collected at 30 min after the galactose dose and a transient change could have been missed. In contrast to measurements of red blood cell galactose-1-phosphate, which are, of course, much more readily available, our studies follow the changes in metabolite levels in the liver that are thought to lead directly to the toxic effects of galactose. ^{31}P MRS is relatively insensitive for detecting changes in galactosemia, but further studies in newly diagnosed patients may help to elucidate the relation between red blood cell galactose-1-phosphate and hepatic levels of this metabolite.

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