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Studies on the Mechanism of Galactosamine Hepatitis: Accumulation of Galactosamine-1-Phosphate and its Inhibition of UDP-Glucose Pyrophosphorylase

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D-Galactosamine has been shown to produce a liver damage closely related to human viral hepatitis. D-Galactosamine-1-phosphate and UDP-galactosamine were identified as the predominant early metabolites of galactosamine in rat liver. The conversion of galactosamine-1-phosphate to UDP-galactosamine is shown to be catalyzed by UDP-glucose: α-D-galactose-1-phosphate uridylyltransferase. The low affinity of this enzyme for galactosamine-1-phosphate explains in part the high levels of this compound found in galactosamine treated livers. Under these conditions galactosamine-1-phosphate accumulation is enhanced by the strongly reduced levels of UDPG. Galactosamine-1-phosphate inhibits the UDPG-pyrophosphorylase reaction, the type of inhibition being mainly competitive with glucose-1-phosphate. In the presence of the concentrations of galactosamine-1-phosphate and glucose-1-phosphate found *in vivo* after galactosamine treatment, UDPG-pyrophosphorylases from rat and calf liver are strongly inhibited *in vitro*. By these mechanisms galactosamine-1-phosphate counteracts its own conversion to UDP-galactosamine. The influence of the strongly diminished UDPG levels on the UDPG-linked syntheses of glycogen, heteropolysaccharides and glucuronides as well as the trapping of uridine phosphates by formation of UDP-hexosamines may play an important role in the induction of galactosamine hepatitis.

We have shown previously that D-galactosamine specifically induces an injury of the liver which closely resembles human viral hepatitis in its morphologic and functional features [1,2]. In order to learn about the mechanism of galactosamine toxicity its metabolism in liver was investigated.

Enzymes. Uridine diphosphoglucose pyrophosphorylase, or UTP:α-D-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9); uridine diphosphoglucose:α-D-galactose-1-phosphate uridylyltransferase (EC 2.7.7.12); amyloglucosidase (from *Aspergillus niger*, splitting α-D-(1→4) and α-D-(1→6) glucosyl bonds, or α-glucanglucohydrolase [8]; galactose dehydrogenase, or D-galactose:NAD oxidoreductase (EC 1.1.1.48); uridine diphosphoglucose dehydrogenase, or uridine diphosphoglucose:NAD oxidoreductase (EC 1.1.1.22); nucleoside diphosphokinase, or ATP:nucleosidediphosphate phosphotransferase (EC 2.7.4.6); phosphoglucomutase, or α-D-glucose-1,6-diphosphate:α-D-glucose-1-phosphate phosphotransferase (EC 2.7.5.1); glucose-6-phosphate dehydrogenase, or D-glucose-6-phosphate:NADP oxidoreductase (EC 1.1.1.49); galactokinase, or ATP:D-galactose-1-phosphotransferase (EC 2.7.1.6); alkaline phosphatase, or orthophosphoric monoester phosphohydrolase (EC 3.1.3.1); glycogen synthetase, or UDP-glucose:α-1,4-glucan α-4-glucosyltransferase (EC 2.4.1.11); phosphodiesterase, or orthophosphoric diester phosphohydrolase (EC 3.1.4.1); NADPH oxidase, or reduced-NADP:(acceptor) oxidoreductase (EC 1.6.99.1); glucose dehydrogenase, or β-D-glucose:NAD (P) oxidoreductase (EC 1.1.1.47).

In earlier studies [3—5] in rats and in the perfused rat liver relatively small amounts of ^{14}C-labelled galactosamine were administered and no liver damage was observed. After liver perfusion with [1-^{14}C]galactosamine several acid-soluble metabolites were identified [5] including galactosamine-1-phosphate, UDP-galactosamine, UDP-glucosamine, UDP-*N*-acetyl-galactosamine and UDP-*N*-acetylglucosamine. Whereas the formation of galactosamine-1-phosphate by galactokinase is well established [6,7], no evidence had been presented for the following enzymic steps in mammalian liver. After administering intraperitoneally a small dose (45 mg/kg) of labelled galactosamine, White *et al.* [4] found radioactive material in the liver decreasing during the first 6 hours. This decrease was not observed by Kuhn *et al.* [3] who injected intraperitoneally 530 mg [^{14}C]galactosamine-1-phosphate · HCl/kg body weight and recovered during the first hours most of the radioactivity from the liver.

We will present evidence that application of more than 200 mg galactosamine · HCl/kg body weight leads to an accumulation of galactosamine-1-phosphate with subsequent inhibition of galactosamine metabolism in liver. It will also be shown that galactos-

amine-1-phosphate is slowly converted to UDP-galactosamine by UDPG:galactose-1-phosphate uridylyltransferase [Eqn. (1)].

$$\alpha\text{-D-GalN-1-}P + \text{UDPG} \rightleftarrows \text{UDPGalN} + \alpha\text{-D-Glc-1-}P \quad (1)$$

Concentrations of galactosamine-1-phosphate found in livers of galactosamine-treated animals are shown to inhibit the enzyme UDPG-pyrophosphorylase [Eqn. (2)],

$$\text{UTP} + \alpha\text{-D-Glc-1-}P \rightleftarrows \text{UDPG} + PP_i \quad (2)$$

which results in a reduced rate of UDPG-synthesis. Greatly decreased levels of this compound observed in galactosamine treated rat livers in turn diminish the rate of galactosamine-1-phosphate conversion to UDP-galactosamine according to equation (1).

MATERIALS

Chemicals and Enzymes

D-Galactosamine · HCl (puriss.) was obtained from C. Roth (Karlsruhe), pentothal as Nembutal® from Abbott GmbH (Frankfurt). Galactose adapted yeast was obtained from Sigma Chem. Comp. (St. Louis, U.S.A.).

The enzymes amyloglucosidase (10 U/mg), galactose dehydrogenase (50 U/mg), UDPG:galactose-1-phosphate uridylyltransferase (1 U/mg) and UDPG dehydrogenase (1 U/mg) containing UDPG pyrophosphorylase (0.25 U/mg) and nucleoside diphosphokinase (0.15 U/mg) were a gift from the Biochemical Division of Boehringer Mannheim GmbH (Mannheim). All other enzymes and coenzymes were purchased from Boehringer Mannheim GmbH (Mannheim).

Animals

Female rats (120—170 g) from the Wistar strain were used throughout. They were fed an unrestricted commercial diet (Altromin® from Altromin GmbH, Lage/Lippe).

METHODS

Galactosamine was injected intraperitoneally as a 0.45 M aqueous solution. The standard procedure consisted of 6 injections of 250 mg galactosamine · HCl/kg body weight each within 24 hours as described previously [1]. In some experiments 250 mg galactosamine · HCl/kg were given only once. Livers were removed under pentothal anaesthesia (45 mg/kg, i. p.) after freezing *in situ* between clamps cooled with liquid nitrogen. The tissue samples were deproteinized with 4 volumes 0.9 N perchloric acid. Glucose from glycogen was determined in the $KHCO_3$-neutralized homogenate after enzymatic hydrolysis with amyloglucosidase [9]. All other metabolites were determined in the neutralized supernatants of the perchloric acid extracts.

UDPG was assayed with UDPG-dehydrogenase at pH 8.7 in the presence of 2 mM NAD and 1 mM EDTA [9]. (In earlier work UDPG dehydrogenase preparations of a lesser purity were used without addition of EDTA [1]. Consistently too high values for UDPG are obtained under these conditions.) For the sequential determination of glucose-1-phosphate or of UTP an excess of UTP and of glucose-1-phosphate respectively (final concentration 1 mM) and magnesium acetate (3 mM) was added to active the UDPG pyrophosphorylase. UDP was determined in the same assay by further addition of nucleoside diphosphokinase from beef liver, which phosphorylates UDP to UTP in the presence of ATP. Alternatively UDPG was assayed using UDPG:galactose-1-phosphate uridylyltransferase in the presence of galactose-1-phosphate (1 mM), phosphoglucomutase, glucose-6-phosphate dehydrogenase and NADP. Galactosamine-1-phosphate was determined in a combined assay using galactose dehydrogenase [15], 2 mM NAD and alkaline phosphatase at pH 8.7. Galactosamine standards were run simultaneously and readings corrected for blanks omitting NAD. Alternatively galactosamine-1-phosphate was assayed in the presence of UDPG:galactose-1-phosphate uridylyltransferase and excess UDPG according to Eqn. (1) and the glucose-1-phosphate formed was determined as in the UDPG assay with UDPG:galactose-1-phosphate uridylyltransferase. Inorganic phosphate was estimated enzymatically as described by Schulz *et al.* [10]. The determinations of the other metabolites have been described previously [1]. The data are given as mean values from different animals ± the standard deviation (S.D.). Enzyme activities are given in international units (U = μmoles $\times \text{min}^{-1}$). Protein was determined with the Biuret method [11].

Preparation of Galactosamine-1-Phosphate

Galactosamine-1-phosphate was synthesized enzymatically using galactokinase from galactose adapted yeast according to Carlson *et al.* [12]. The yield of galactosamine-1-phosphate was increased to more than 90 % by using twice the amount of ATP and 10 mM sodium fluoride. In addition to chromatography on Dowex-50 (H^+) the product was further purified on Dowex-1 formate (elution with 0.8 M formic acid). After lyophilisation and precipitation by 80 % ethanol crystals of the ammonium salt formed under reduced pressure. Galactosamine-1-phosphate was determined by the modified Elson-Morgan reaction after acetylation and acid hydrolysis [12] and alternatively by using alkaline phosphatase and galactose dehydrogenase. The optical rotation

$[\alpha]_D^{25} = 125°$ ($c = 1.93$ in H_2O) for galactosamine-1-phosphate ammonium salt was in good agreement with the value obtained by Carlson *et al.* [12]. *N*-Acetylgalactosamine-1-phosphate was prepared by acetylation of galactosamine-1-phosphate [12].

Measurements of Enzyme Activities

All activities were measured at 25° using a recording Eppendorf photometer. The assay system of UDPG:galactose-1-phosphate uridylyltransferase (1 U/mg) consisted in a final volume of 0.70 ml of 150 mM triethanolamine pH 8.7, 1.5 mM magnesium acetate, 0.7 mM UDPG, 0.7 mM NADP, 70 mM mercaptoethanol, 10 μM glucose-1,6-diphosphate, galactosamine-1-phosphate and galactose-1-phosphate as indicated, 160 μg phosphoglucomutase (100 U/mg), 80 μg glucose-6-phosphate dehydrogenase (140 U/mg), 50 μg UDPG:galactose-1-phosphate uridylyltransferase (1 U/mg). A more than 1000-fold excess of the indicator enzymes did not alter the reaction velocities. The UDPG:galactose-1-phosphate uridylyltransferase used contained less than 0.01% phosphodiesterase, less than 0.1% NADPH oxidase, less than 1% glucose dehydrogenase and less than 5% UDPG pyrophosphorylase [data from Boehringer Mannheim GmbH (Mannheim)].

If UDP-galactosamine or UDP-galactose were substrates of the reaction the assay contained, besides buffer and mercaptoethanol, 0.7 mM glucose-1-phosphate, 2 mM NAD and UDP-glucose dehydrogenase as the indicator enzyme. K_m and V_{max} values were computed from Lineweaver-Burk plots by the least squares method.

UDP-glucose pyrophosphorylase was assayed by the following methods: (a) Glucose-1-phosphate formation from UDPG in the presence of pyrophosphate according to Hansen *et al.* [13]. (b) Conversion of glucose-1-phosphate and UTP to UDPG using an enzyme mixture of UDPG dehydrogenase and UDPG pyrophosphorylase from beef liver. The assay system consisted of 180 mM triethanolamine pH 8.7, 1.8 mM $MgCl_2$, 0.8 mM UTP, 0.3—0.8 mM glucose-1-phosphate, and 2 mM NAD. (c) Most of the kinetic experiments were conducted in 30 mM Tris-buffer pH 7.4 in the presence of 2 mM magnesium acetate, 0.4 to 1.4 mM UTP, and glucose-1-phosphate as indicated and UDPG pyrophosphorylase from rat liver (100 μl); the final volume was 0.7 ml; the mixture was incubated for 2 min at 25° in stoppered tubes and the reaction discontinued by heating the tubes for 3 min in a boiling water bath. After cooling UDPG was determined in an aliquot as described above.

Preparation of Rat Liver UDPG Pyrophosphorylase

The enzyme was prepared from rat livers (850 g) in a similar way as described by Hansen *et al.* [15] for the calf liver enzyme. Protamine sulfate precipitation of the tissue extract, ammonium sulfate (40—58%) fractionation, calcium phosphate gel treatment, chromatography on DEAE-cellulose and a second ammonium sulfate precipitation were carried out as described [13]. After dialysis against 0.02 M triethanolamine buffer pH 8.5 (5 μM UDPG, 10 μM pyrophosphate) the enzyme preparation was kept at −20° for at least 24 hours. Inactive protein was then removed by centrifugation at $2000 \times g$ for 10 min. The overall purification at this step was at least 50-fold over the crude extract.

RESULTS

Liver Metabolites after Galactosamine Application in vivo

After injection of the standard dose of galactosamine (6×250 mg galactosamine · HCl/kg) highly significant decreases of several metabolites in the liver were observed (Table 1). Whereas the levels of free glucose, glucose-6-phosphate and glucose-1-phosphate are reduced to 30—50%, the glycogen content is as low as 5% of the control values. The concentration of UDPG, the substrate for glycogen synthesis, decreased to about 16% of normal. Not only the carbohydrate metabolites but also the levels of uridine and adenine nucleotides were significantly changed (Table 1). However, the ratio ATP/ADP was not much altered: 2.96 ± 0.66 in galactosamine treated animals *vs.* 3.36 ± 0.55 in the controls. The "energy charge" according to Atkinson and Walton [14] [(ATP + 1/2 ADP):(ATP + ADP + AMP)] was 0.82 and 0.81 respectively; neither was the ratio glucose-6-phosphate/glucose affected (0.020 ± 0.015 *vs.* 0.016 ± 0.006) [1].

The time dependent alterations of some liver metabolite levels were best observed after a single injection of 250 mg galactosamine · HCl/kg (Fig. 1). A pronounced drop of UDPG takes place soon after the injection, followed by the decrease of the glycogen content.

Galactosamine-1-Phosphate in the Liver

Attempts to analyze specifically the concentrations of galactosamine in liver were undertaken using an assay with galactose dehydrogenase from *Pseudomonas saccharophila* [15]. Since the rate of dehydrogenation of galactosamine is only 1% (at 20 mM) of that of D-galactose, it is possible to differentiate clearly between galactosamine and galactose. Under these conditions neither free galactose nor galactosamine could be found in appreciable amounts in liver extracts of normal or galactosamine treated rats. However, after addition of alkaline phosphatase to the assay galactosamine became detectable in the samples from the galactosamine-damaged livers (10.7 ± 0.9 μmoles/g) but not in the controls

Table 1. *Liver metabolites*
25.5 hours after the first and 1.5 hours after the last of 6 galactosamine-injections (250 mg galactosamine · HCl/kg each) the rat livers were frozen *in situ*. Mean values (μmoles/g fresh liver), the standard deviation (S.D.) and the number of animals (n) are shown below. All differences are highly significant, except for inorganic phosphate

	Control			Galactosamine-treated			
	Mean	S.D.	n	Mean	S.D.	n	Compared to control
	μmoles/g			μmoles/g			%
Glycogen (glucosyl units)	338	±62	14	16	±9	12	5
Glucose	8.8	± 0.7	11	3.8	±1.0	11	43
UDPG	0.32	± 0.05	42	0.05	±0.01	9	16
Glc-1-*P*	0.06	± 0.02	10	0.02	±0.01	4	33
GalN-1-*P*	< 0.04	—	6	10.7	±0.9	6	—
UTP + UDP	0.35	± 0.05	15	0.15	±0.02	4	43
ATP + ADP + AMP	3.23	± 0.30	7	2.23	±0.42	9	69
P_i	3.18	± 0.56	9	3.43	±0.33	8	—

Table 2. *Kinetic properties of UDPG:galactose-1-phosphate uridylyltransferase from calf liver*
For experimental conditions see Methods

Substrate	[S]	V	K_m	V_{max}
	mM	μmoles/min	mM	μmoles/min
Gal-1-*P*	1	100	0.17	100
GalN-1-*P*	1	7.5	15	135
GalNAc-1-*P*	1	0.2	—	—
Gal-1-*P* + GalN-1-*P*	1	270	0.23	270

(Table 1). Similar results were obtained when the supernatants were hydrolyzed with 1 N HCl at 100° for 15 min. Treatment with 0.1 N HCl at 100° liberated within 10 min only 25% of the galactosamine; the same resistance against hydrolysis was observed with an authentic preparation of galactosamine-1-phosphate; it is also in agreement with data of Cardini and Leloir [16].

Further evidence for the identity of this phosphorylated galactosamine derivative with α-D-galactosamine-1-phosphate was obtained by enzymatic conversion with UDPG:galactose-1-phosphate uridylyltransferase from calf liver according to Eqn. (1). *N*-Acetyl-galactosamine-1-phosphate was excluded by the above methods, because it is much more acid labile [12], and it is converted to UDP-*N*-acetyl-galactosamine by UDPG:galactose-1-phosphate uridylyltransferase with only about 2% of the rate of galactosamine-1-phosphate (Table 2); furthermore *N*-acetyl-galactosamine-1-phosphate, after hydrolytic removal of its phosphate group is not oxidized by galactose dehydrogenase.

2.6 μmoles galactosamine-1-phosphate/g liver were found 1 hour after the intraperitoneal application of 1×250 mg galactosamine-1-phosphate · HCl/kg (Fig. 1).

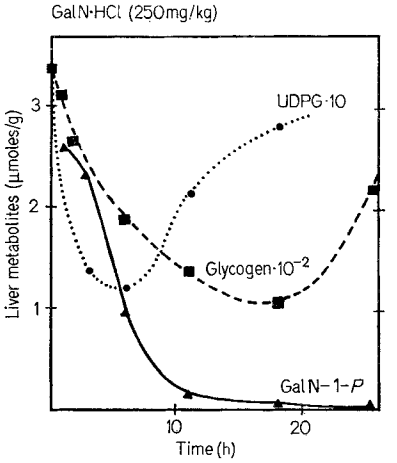

Fig. 1. *Time course of metabolite contents in rat liver.* GalN-1-*P*, UDPG and glycogen contents (μmoles/g) were determined after a single injection of 250 mg GalN · HCl/kg. Each point is the mean from at least 3, usually 6, rat livers

Inhibition of UDP-Glucose Pyrophosphorylase by Galactosamine-1-Phosphate

Since galactosamine treatment of rats results in a drastic reduction of the UDPG and glycogen levels in liver, and since it was shown that galactosamine-1-phosphate and not galactosamine accumulates in this organ galactosamine-1-phosphate was tested as inhibitor of UDPG-pyrophosphorylase from rat and beef liver. Experiments with varying concentrations of glucose-1-phosphate (0.15—1.5 mM) and of galactosamine-1-phosphate (5—15 mM) showed that both enzymes are inhibited by galactosamine-1-phosphate in a predominantly competitive way [17]. In the presence of 14 mM galactosamine-1-phosphate, 0.4 mM UTP and rat liver enzyme a K_i-value of 5 mM was obtained for galactosamine-1-phosphate and a K_m of 0.19 mM for glucose-1-phosphate (Fig. 2);

raising the UTP concentration to 1.4 mM resulted in a lower K_i (3.2 mM) for galactosamine-1-phosphate. Galactosamine-1-phosphate was also found to be an effective inhibitor of the reverse reaction, when UDPG and PP_i were used as substrates and the glucose-1-phosphate formation was followed.

As the inhibition by galactoamine-1-phosphate is mainly competitive with regard to glucose-1-phosphate the ratio of galactosamine-1-phosphate to glucose-1-phosphate in liver after galactosamine application is of primary importance. According to the data of Fig. 2 a ratio (galactosamine-1-phosphate/glucose-1-phosphate) of about 100 decreases the activity of UDPG-pyrophosphorylase to one third of the control value. 1.5 hours after the last and 25.5 hours after the first injection of galactosamine (standard procedure) the galactosamine-1-phosphate/glucose-1-phosphate ratio in the liver is more than

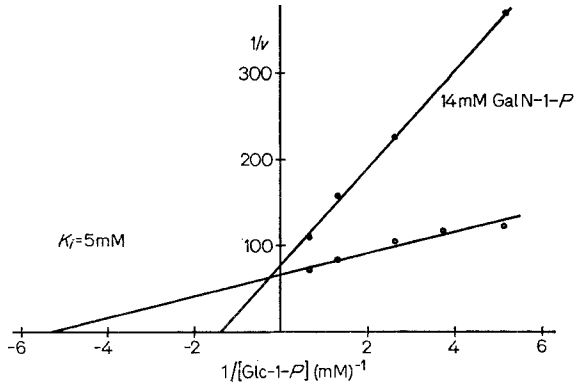

Fig. 2. *Inhibition of rat liver UDPG-pyrophosphorylase by GalN-1-P.* Incubation at 25° for 1 min at pH 7.4 in the presence of 0.4 mM UTP; for further details see Methods

600/1. It can be concluded that UDPG-pyrophosphorylase activity *in vivo* is strongly inhibited at that time; it seems reasonable to attribute the observed effects on glycogen synthesis to the level of galactosamine-1-phosphate. Fig. 1 clearly demonstrates this correlation: UDPG and, with some delay, glycogen synthesis in liver are resumed only after the concentration of galactosamine-1-phosphate has dropped below 1 mM.

Specificity of UDPG: Galactose-1-Phosphate Uridylyltransferase

From the data of Fig. 1 it can be computed that galactosamine-1-phosphate disappears in the liver under the conditions used with a half life time of about 3 hours. Although, after galactosamine application, the appearence of UDP-galactosamine in rat liver was described by Maley *et al.* [5] and was seen in our experiments, the enzymatic pathway of UDP-galactosamine formation has not yet been established. UDPG:galactose-1-phosphate uridylyltransferase activity in the supernatant fractions (35000 × *g*, 10 min) as measured with galactose-1-phosphate and UDPG as substrates was not inhibited in galactosamine-treated livers nor by the addition of 1 mM galactosamine-1-phosphate. In fact, it could be shown that galactosamine-1-phosphate is a substrate of purified UDPG:galactose-1-phosphate uridylyltransferase from calf liver [Eqn. (1)].

The data of Table 2 show that galactosamine-1-phosphate at high concentrations is converted to the UDP derivate even faster than galactose-1-phosphate, but the affinity of the enzyme is considerably lower for galactosamine-1-phosphate. Using [1-^{14}C]-galactosamine-1-phosphate the radioactive UDP-galactosamine was separated by twice repeated paper chromatography (ethanol—1 M ammonium acetate, 15:6, v/v, pH 7.5). The formation of UDPG by UDPG:galactose-1-phosphate uridylyltransferase in the presence of labelled UDP-galactosamine and glucose-1-phosphate was assayed by NAD reduction after the addition of UDPG dehydrogenase. At a concentration of 2 mM galactosamine-1-phosphate 15 % of the maximal velocity with galactose-1-phosphate is reached. Under these conditions about 6 μmoles of UDP-galactosamine would be formed by UDPG:galactose-1-phosphate uridylyltransferase in 1 hour per 1 g of rat or calf liver.

In the presence of saturating concentrations of galactose-1-phosphate activation of UDPG:galactose-1-phosphate uridylyltransferase by galactosamine-1-phosphate (up to 1 mM) was observed (Table 2). Using the Lineweaver and Burk plot the half maximal activation was calculated at 33 μM galactosamine-1-phosphate.

With high enzyme concentrations (1 mg/ml) *N*-acetyl-galactosamine-1-phosphate was seen to be another possible substrate for UDPG:galactose-1-phosphate uridylyltransferase. In the presence of 1 mM *N*-acetyl-galactosamine-1-phosphate 0.2 % of the velocity with galactose-1-phosphate was determined.

DISCUSSION

The biochemical processes involved in the hepatitis-like liver damage caused by galactosamine may elucidate a new mechanism of liver toxicity. At present nothing is known about the biochemistry of viral hepatitis; therefore, no conclusions can yet be drawn about correlations in the molecular mechanisms, although many functional and morphologic similarities have been observed between human viral and galactosamine hepatitis [1].

The rapid phosphorylation of galactosamine by liver galactokinase and the low affinity of UDPG:galactose-1-phosphate uridylyltransferase for galactosamine-1-phosphate explain in part the high levels of galactosamine-1-phosphate in galactosamine-

treated liver. As UDPG is a necessary substrate for the UDPG:galactose-1-phosphate uridylyltransferase reaction leading to glucose-1-phosphate and UDP-galactosamine [Eqn. (1)], the UDPG depletion due to UDPG-pyrophosphorylase inhibition by galactosamine-1-phosphate, further augments the accumulation of galactosamine-1-phosphate. By this mechanism galactosamine-1-phosphate inhibits its own conversion to UDP-galactosamine. Consequently the rate of galactosamine-1-phosphate metabolism is dependent on the galactosamine dose applied (Fig. 3). This explains the difference between the experiments of Kuhn *et al.* [3] and White *et al.* [4] mentioned in the introduction.

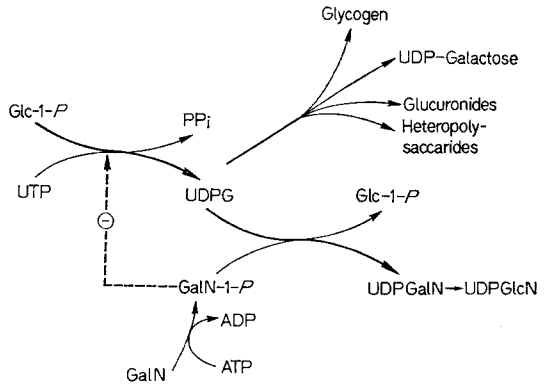

Fig. 3. *Inhibition of UDPG-pyrophosphorylase and UDPG dependent biosyntheses by GalN-1-P in liver*

In 1968 a pathway for galactosamine metabolism in rat liver was proposed by Maley *et al.* [5]: galactosamine → *N*-acetyl-galactosamine → *N*-acetyl-galactosamine-1-phosphate → UDP-*N*-acetyl-galactosamine. The acetylation reaction studied in pigeon liver [18], is rather sluggish (about 30 pmoles × min^{-1} × mg $protein^{-1}$) and appears to be the limiting step in this sequence. In comparison, the galactokinase and UDPG:galactose-1-phosphate uridylyltransferase reaction velocities are more than 100 times faster. Furthermore the formation of considerable amounts of UDP-galactosamine and UDP-glucosamine in rat liver after galactosamine-application cannot be explained by the acetylation pathway. According to our results and to the reactions described earlier [6,7,19] the following scheme for galactosamine metabolism in rat and calf liver can be given: galactosamine $\xrightarrow{ATP}$ galactosamine-1-phosphate $\xrightarrow{UDPG}$ UDP-galactosamine → UDP-glucosamine (Fig. 3). Enzymatic reactions leading from UDP-glucosamine to UDP-*N*-acetyl-galactosamine (*via* glucosamine-1-phosphate and UDP-*N*-acetyl-glucosamine) were described already (summarized by [20]).

The inhibition of the UDPG-pyrophosphorylase reaction by galactosamine-1-phosphate reminds one of the effect of galactose-1-phosphate on this enzyme; the latter was discussed as an important mechanism of galactose toxicity in galactosaemia [21]. Whereas the accumulation of galactose-1-phosphate in galactosaemia is due to a lack of UDPG-galactose-1-phosphate uridylyltransferase [22], the accumulation of galactosamine-1-phosphate in galactosamine-hepatitis is mainly due to the low affinity of this enzyme for galactosamine-1-phosphate.

The conclusion that the inhibition of UDPG-pyrophosphorylase is also effective *in vivo* is supported by the high ratio of galactosamine-1-phosphate/glucose-1-phosphate in the galactosamine-damaged liver and by the time course of metabolite contents in liver after one galactosamine injection.

The influence of the diminished levels of uridine (and adenine) nucleotides on UDPG and other uridine phosphate dependent syntheses cannot be evaluated quantitatively at the present time. One may suppose that the decrease of normally occurring uridine nucleotides (UTP, UDP, UDPG) is accompanied by increased amounts of UDP-hexosamines arising from galactosamine-1-phosphate. It is surprising that no reduction of the inorganic phosphate pool due to excess galactosamine-1-phosphate and UDP-hexosamine formation is observed.

A diminished UDPG synthesis in turn influences the pathways leading to UDP-galactose, UDP-glucuronic acid, glycogen and heteropolysaccharides. The increased serum bilirubin concentrations in galactosamine treated animals suggest that glucuronidation is affected. If and to what extent the biosynthesis of heteropolysaccharides and the detoxication by glucuronidation is influenced by the strongly diminished UDPG formation is subject to further work. The glycogen depletion of galactosamine treated livers can also be explained by the diminished UDPG levels; according to the data of Mersmann and Segal [23] the rate of glycogen synthetase *a* is reduced to less than 25 % when the UDPG concentration decreases from 0.32 to 0.05 mM (Table 1). The almost complete exhaustion of the glycogen store within 25.5 hours in galactosamine-treated livers could be due to the inhibition of synthesis or to stimulation of degradation or both. During perfusion of isolated galactosamine damaged livers no increase of glycogen breakdown and glucose release as compared with normal livers was observed [24]. Although an enhancement of glycogenolysis *in vivo* to some extent cannot be ruled out completely, the reduction of the glycogen content after galactosamine treatment appears to be due mainly to a reduced synthetic rate.

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REFERENCES

1. Keppler, D., Lesch, R., Reutter, W., and Decker, K., *Exptl. Mol. Pathol.* 9 (1968) 279.
2. Reutter, W., Lesch, R., Keppler, D., and Decker, K., *Naturwissenschaften*, 55 (1968) 497.
3. Kuhn, R., Leppelmann, H. J., and Fischer, H., *Liebigs Ann. Chem.* 620 (1959) 15.
4. White, B. N., Shetlar, M. R., Shurley, H. M., and Schilling, J. A., *Biochem. Biophys. Acta*, 101 (1965) 259.
5. Maley, F., Tarentino, A. L., McGarrahan, J. F., and Del Giacco, R., *Biochem. J.* 107 (1968) 637.
6. Ballard, F. J., *Biochem. J.* 98 (1966) 347.
7. Walker, D. G., and Khan, H. H., *Biochem. J.* 108 (1968) 169.
8. Pazur, J. H., and Ando, T., *J. Biol. Chem.* 235 (1960) 297.
9. Keppler, D., and Decker, K., in *Methoden der enzymatischen Analyse*, 2. Aufl. (edited by H. U. Bergmeyer), Verlag Chemie, Weinheim 1969 (in press).
10. Schulz, D. W., Passoneau, J. V., and Lowry, O. H., *Anal. Biochem.* 19 (1967) 300.
11. Beisenherz, G., Boltze, H. J., Bücher, Th., Czok, R., Garbade, K. H., Meyer-Arendt, E., and Pfleiderer, G., *Z. Naturforsch.* 8b (1953) 555.
12. Carlson, D. M., Swanson, A. L., and Roseman, S., *Biochemistry*, 3 (1964) 402.
13. Hansen, R. G., Albrecht, G. J., Bass, S. T., and Seifert, L. L., *Methods in Enzymology* (edited by S. P. Colowick and N. O. Kaplan), Academic Press, New York and London 1966, Vol. VIII, p. 248.
14. Atkinson, D. E., and Walton, G. M., *J. Biol. Chem.* 242 (1967) 3239.
15. Wallenfels, K., and Kurz, G., *Biochem. Z.* 335 (1962) 559.
16. Cardini, C. E., and Leloir, L. F., *Arch. Biochem. Biophys.* 45 (1953) 55.
17. Keppler, D., and Decker, K., *Hoppe-Seyler's Z. Physiol. Chem.* 350 (1969) 8.
18. Chou, T. C., and Soodak, M., *J. Biol. Chem.* 196 (1952) 105.
19. Maley, F., and Maley, G. F., *Biochem. Biophys. Acta*, 31 (1959) 577.
20. Fischer, W., and Weinland, H., *Stoffwechsel der Galaktose und ihrer Derivate*, Thieme Verlag, Stuttgart 1965.
21. Oliver, I. T., *Biochem. Biophys. Acta*, 52 (1961) 75.
22. Anderson, E. P., Kalckar, H. M., and Isselbacher, K. J., *Science*, 125 (1957) 113.
23. Mersmann, H. J., and Segal, H. L., *Proc. Natl. Acad. Sci. U. S.* 58 (1967) 1688.
24. Fröhlich, J., Keppler, D., Reutter, W., Decker, K., and Wieland, O., unpublished observations.

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