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Research Article

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Basis for the Control of Purine Biosynthesis by Purine Ribonucleotides

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ABSTRACT An animal model was used to determine the basis for the increase in purine biosynthesis that results from hepatic depletion of purine nucleotides, such as seen in patients with type I glycogen storage disease or following fructose administration. Mice were injected intravenously with glucose or fructose, 2.5 mg/g of body weight, and the animals were killed at 0, 3, and 30 min following carbohydrate infusion. Fructose, but not glucose, administration led to a threefold increase in [^{14}C]glycine incorporation into hepatic purine nucleotides documenting an increase in the rate of purine biosynthesis in the liver of fructose-treated animals. In the fructose, but not the glucose-treated animals, there was a reduction in the hepatic content of purine nucleotides that are inhibitory for amidophosphoribosyltransferase, the enzyme that catalyzes the first reaction unique to the pathway of purine biosynthesis. PP-ribose-P, an important metabolite in the control of purine biosynthesis, was increased 2.3-fold in liver following fructose, but not glucose administration. In conjunction with the decrease in inhibitory nucleotides and increase in PP-ribose-P 29% of amidophosphoribosyltransferase was shifted from the large inactive to the small active form of the enzyme. Results of these studies demonstrate that the end-products of the pathway, purine nucleotides, control the activity of the enzyme that catalyzes the first reaction leading to purine nucleotide synthesis either through a direct effect of purine nucleotides on the enzyme, through an indirect effect of the change in nucleotides on PP-ribose-P synthesis, or a combination of these effects. The resultant changes in amidophosphoribosyltransferase conformation and activity provide a basis for understanding the increase in purine biosynthesis that results from hepatic depletion of purine nucleotides.

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INTRODUCTION

Hyperuricemia is seen in patients with type I glycogen storage disease and in normal subjects receiving fructose, and it is associated with an increase in the rate of purine biosynthesis *de novo* (1–8). The increase in purine biosynthesis in both of these conditions is thought to be a response on the part of the liver to an acceleration in the rate of purine nucleotide catabolism. Following fructose administration purine nucleotide catabolism is accelerated as a consequence of ATP utilization for phosphorylation of fructose and decrease in inorganic phosphate content of liver (9). The decrease in ATP and inorganic phosphate leads to the activation of enzymes required for the degradation of AMP to nucleosides, bases, and ultimately, uric acid. In patients with type I glycogen storage disease the stimulus to glycogen catabolism brought on by fasting results in the accumulation of glucose-6-phosphate as a consequence of the deficiency of glucose-6-phosphatase. The inability to hydrolyze glucose-6-phosphate in these patients leads to a reduction in hepatic content of inorganic phosphate and a reduction in ATP as the glucose-6-phosphate is metabolized through the Embden-Meyerhof pathway (10). As with fructose administration, the depletion of ATP and inorganic phosphate sets the stage for catabolism of AMP to uric acid in type I glycogen storage disease.

The mechanism by which purine nucleotide depletion leads to an increase in the rate of purine biosynthesis in type I glycogen storage disease and after fructose administration is not known. To get to the basis for accelerated purine synthesis under these conditions it is necessary to biopsy liver at different points in time for quantification of metabolites and enzymes that play a role in the control of this pathway. Since repeated biopsies of human liver are not feasible, an animal model was selected for this study in which nucleotide catabolism was induced by intravenous infusion of fructose. Using this model it was possible to obtain

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liver samples at different points in time for the quantification of PP-ribose-P and purine nucleotides. Intracellular concentrations of PP-ribose-P and purine nucleotides are important determinants of the rate of purine biosynthesis (11, 12). In addition, the animal model selected for these studies provided tissue for the determination of the relative distribution of amidophosphoribosyltransferase between the active and inactive forms of the enzyme. The latter studies are potentially important, since prior work has demonstrated that the interconversion of this enzyme, which catalyzes the first reaction unique to the pathway of purine synthesis, between its active and inactive forms is controlled by the relative concentrations of PP-ribose-P and purine nucleotides (13). Using this experimental approach it has been possible to develop a model that explains at the enzymatic level the basis for accelerated purine biosynthesis that results from depletion of hepatic purine nucleotides.

METHODS

Animals and materials. Male CD-1 mice between the ages of 4 and 7 wk were purchased from Charles River Breeding Laboratories, Wilmington, Mass. Uniformly labeled L-[^{14}C]-glutamine (49 mCi/mmol) was purchased from New England Nuclear, Boston, Mass. Sodium salts of phosphoribosylpyrophosphate (PP-ribose-P), AMP, ADP, ATP, guanosine 5′-monophosphate (GMP), guanosine 5′-diphosphate (GDP), guanosine 5′-triphosphate (GTP), uridine 5′-triphosphate (UTP), cytidine 5′-triphosphate (CTP), inosine 5′-monophosphate (IMP), dithiothreitol, Trizma HCl, bovine serum albumin, and tri-*N*-octylamine were purchased from Sigma Chemical Co., St. Louis, Mo. Catalase was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wisc. Sucrose and anhydrous granular glucose were purchased from MCB Reagents, Cincinnati, Ohio. CM-Sephadex and DE-52 were purchased from Whatman Inc., Clifton, N. J. Ultrogel AcA 34 was purchased from LKB Instruments, Inc., Rockville, Md.

Enzyme assay. The amidophosphoribosyltransferase assay was performed in a total volume of 100 μl of 50 mM KPi buffer, pH 7.4, with 5 mM $MgCl_2$, 5 mM PP-ribose-P, 4 mM [^{14}C]glutamine, and 5 mM dithiothreitol as previously described (14). The total amount of protein added to the assay depended upon the purity of the enzyme preparation used and varied from 0.30 to 3.9 mg. The use of 50 mM Pi in the assay provided maximal activation of glutaminase in mouse liver extract, similar to results obtained in rat liver extract (15). Amidophosphoribosyltransferase activity represented the difference between [^{14}C]glutamate formation in the presence and absence of PP-ribose-P. In mouse liver extract glutaminase activity, i.e., glutamine hydrolysis in the absence of PP-ribose-P, was approximately equal to amidophosphoribosyltransferase activity, i.e., PP-ribose-P dependent glutamine hydrolysis. Heat inactivation of glutaminase (15) gave similar results for amidophosphoribosyltransferase activity in crude extract, but this procedure could not be routinely used for experiments designed to determine amidophosphoribosyltransferase molecular size because of catabolism of PP-ribose-P and purine ribonucleotides in the extract under these conditions.

Enzyme purification. Mouse liver was homogenized with a Dounce tissue grinder in 1 vol of 25 mM potassium phosphate (KPi) buffer, pH 7.4, which contained 5 mM $MgCl_2$ and 15 mM 2-mercaptoethanol. After centrifugation at 10,000 *g* for 30 min at 4°C, the liver homogenate was applied to a DE-52 column equilibrated with the above buffer and eluted with a linear gradient of 50–500 mM KCl as previously described (16). This partial purification resulted in recovery of 63% of the starting activity, and yielded an amidophosphoribosyltransferase preparation free of glutaminase and nucleotidase activities. This preparation produced 125 nmol glutamate/h per mg protein and represented a 5.7-fold purification of amidophosphoribosyltransferase. The partially purified enzyme was used to determine the relative potency of the different nucleotides as inhibitors of mouse liver amidophosphoribosyltransferase and to characterize the physical properties of this enzyme.

Animal model. Mice were kept on an alternating schedule of light and dark, 12 h each, and allowed food and water ad lib. All animals were killed at approximately the same time each morning to avoid potential changes attributable to diurnal variation in metabolites (17, 18). Animals to be injected with carbohydrate received 2.5 mg/g of body weight of fructose or glucose through the tail vein as a 20% solution in 0.9% NaCl over a 10-s period of time. Animals were killed at 3 or 30 min following the intravenous infusion of carbohydrate. Control animals did not receive an intravenous infusion. 1.5 min before death the animals were anesthetized with ether, the abdominal cavity opened while the animal was well oxygenated, and the most peripheral portion of the liver frozen between metal tongs precooled in liquid nitrogen. This piece of liver, 10–60 mg wet wt, was kept under liquid nitrogen (<30 min) until the tissue could be processed for determination of PP-ribose-P, nucleotides, or amidophosphoribosyltransferase molecular form.

Preparation of liver extracts. (*a*) To prepare extract for amidophosphoribosyltransferase assay and determination of relative distribution between the large and small forms of the enzyme freeze-clamped samples from mouse liver were pulverized under liquid nitrogen and then mixed with 1.5 vol of 25 mM KPi buffer, pH 7.4, which contained 15 mM 2-mercaptoethanol and 20% ethylene glycol. This extraction buffer was chilled to −20°C before mixing with the liver powder, and the mixture of buffer and powdered liver was maintained at this temperature in a −20°C cold room. After centrifugation at −20°C for 5 min in a Beckman microfuge (Beckman Instruments, In., Spinco Div., Palo Alto, Calif.), the supernatant fluid was immediately applied to a gel filtration column which was developed at 4°C as described below. Preparation of the extract at −20°C was necessary to prevent catabolism of PP-ribose-P and purine nucleotides before the enzyme sample was applied to the gel filtration column. Over 70% of the amidophosphoribosyltransferase activity in liver extract was recovered from the gel filtration step, and the recovery of amidophosphoribosyltransferase activity was comparable for the extract obtained from the liver of fructose and glucose treated animals. (*b*) To prepare liver extract for the determination of PP-ribose-P a modification of the method of Lalanne and Henderson was used (19). Freeze-clamped mouse liver was pulverized under liquid nitrogen, and the powder (<10 mg of protein) transferred to a Corex tube (Corning Glass Works, Corning, N. Y.) precooled in liquid nitrogen. To this powder was added 1 ml of boiling 25 mM Tris-HCl buffer, pH 7.4, which contained 1 mM Na EDTA, and this tube was immediately placed in a boiling water bath for 90 s. The tube was then placed in a 4°C ice bath for 5–10 min, and the supernate obtained after centrifugation at 10,000 *g* at 4°C for 30 min was used for the assay of PP-ribose-P as described below. The protein pellet was resuspended in 1.0 ml of Tris buffer and protein content of the sample quantified by the method of Lowry et al. (20). (*c*) For nucleotide extraction freeze-clamped liver (10–30 mg) was homogenized at 4°C in 0.5 ml of 12% trichloro-

acetic acid (TCA) for 30 min followed by centrifugation at 4°C for 2 min at 2,000 *g*. The TCA was removed by extraction with an equal volume of 0.5 M tri-*N*-octylamine in freon. The upper aqueous layer resulting from this separation was used for nucleotide analysis. This solution was clarified by passage through a 0.45-μM filter and stored at −70°C until injected onto the high performance liquid chromatograph. Nucleotides prepared by this method were stable for >3 mo when stored at −70°C.

Sucrose density gradients and gel filtration chromatography. Sucrose density gradients were prepared in 25 mM KPi buffer, pH 7.4, with 15 mM 2-mercaptoethanol (buffer A), and centrifugation performed as previously described (13). 200 μl of enzyme preparation or standard were applied to an 11.8-ml gradient. 3 ml of enzyme preparation or standard were applied to a 1.8 × 95-cm Ultrogel AcA-34 column equilibrated with buffer A. Bovine serum albumin (2.5 mg/ml) and catalase (2.0 mg/ml) were used to calibrate the sucrose density gradients and gel filtration column for determination of s_{20w} and Stokes radii, respectively. Molecular weight was estimated from the s_{20w} and Stokes radius determined by these techniques.

Assay for PP-ribose-P. A modification of the technique described by Lalanne and Henderson (19) was used. 50 μl of extract, prepared as described above, was used in an assay of 120 μl that contained 18.7 mM Tris-HCl, pH 7.4, 2 mM [^{14}C]adenine (42.3 μCi/μM), 75 μg of partially purified adenine phosphoribosyltransferase, 10.4 mM $MgCl_2$ 1.0 mM UTP and 3.0 mM 2,3,-diphosphoglycerate (DPG). Inclusion of 2,3-DPG and UTP was necessary to inhibit residual PP-ribose-P synthetase activity and 5′-nucleotidase activity during the assay for PP-ribose-P. [^{14}C]AMP was separated, identified, and counted as described by Thomas et al. (21). Results are expressed as nanomoles of PP-ribose-P per milligram of liver protein.

Determination of purine ribonucleotides. Separation and quantitation were accomplished using a high performance liquid chromatography system obtained from Waters Associates (Milford, Mass.) and included two model 6000A solvent delivery systems, a dual channel (254 nm/280 nm) model 440 absorbance detector, a model 660 programmer, A U6K septumless injector, and a data module. A Whatman Partisil-10 SAX microparticle (10 μm) anion exchange column (256 mm long × 4.6 mm internal Diam) was used to achieve separation according to the procedure of Rose and Brockman (22). The flow rate was set at 2 ml per min, and the run required 40 min at 1,200 pounds per square inch for development of a linear gradient from 5 mM $NH_4H_2PO_4$ buffer, pH 2.8, to 750 mM $NH_4H_2PO_4$ buffer, pH 3.8. The column was maintained at ambient temperature during the run. Buffers were filtered (0.45-μm pore size membrane filter) prior to use. Nucleotides were identified by relative absorbance at 254/280 nm and elution time in comparison to known standards. The concentration of nucleotide in the extract was determined by integrating the area under the peak recorded at 254 nm and comparing this to the area for known concentrations of each nucleotide. Results are expressed in micromoles per gram of wet liver weight.

[^{14}C]*Glycine incorporation.* To assess the rate of purine biosynthesis *de novo* mice were injected with 5 μCi of [^{14}C]-glycine at the same time glucose or fructose was infused. Animals were killed 30 min later by a sharp blow to the head, the liver excised, and homogenized in 8 ml of 2 N perchloric acid. After centrifugation at 2,000 *g* for 10 min at 4°C, the precipitate was saved for determination of glycine incorporation into protein, and the supernate used for determination of glycine incorporation into purine. The supernate was placed in a boiling water bath for 60 min and then neutralized with 1.2 ml of 17 N NH_4OH. 1 ml of 10% $AgNO_3$ was added, and after thorough mixing the sample was held at 4°C overnight. The next day the sample was centrifuged at 5,000 *g* for 5 min at 4°C, the supernate discarded, and the precipitate washed with 3 ml of H_2O. 2 ml of 1 N HCl were added to the precipitate and the mixture placed in a boiling water bath for 60 min. The samples were then chilled to 4°C, and clarified by centrifugation at 5,000 *g* for 5 min. The purine bases in the supernate were isolated by sequential washes of a 1 × 3-cm Dowex AG50W column (Dow Chemical Co., Midland, Mich.) with 10 ml of 0.1 N HCl, 10 ml of 1.0 N HCl, and 2.5 ml of 6 N HCl. Hypoxanthine and guanine were eluted in the 1.0 N HCl wash, and adenine was eluted in the 6 N HCl wash as determined by chromatography of the respective fractions on cellulose thin-layer plates in butanol/methanol/water/ammonia (60:20:20:1) (23). The ratio of the optical density at 280/260 nm for the 6 N HCl fraction was 0.37, also indicating that adenine was the predominant base in this fraction (24). The adenine content of the 6 N HCl wash was determined spectrophotometrically from the absorbance at 260 nm (24), and the radioactivity of this fraction was determined by liquid scintillation spectrometry. The rate of purine biosynthesis *de novo* in control, glucose, and fructose-treated animals was estimated from the amount of radiolabeled carbon counts per minute incorporated per micromole of adenine.

The protein pellet from the original acid precipitation was washed three times with water and resuspended in 0.05 N NaOH with vigorous mixing. After clarification by centrifugation, the protein content of the supernate was determined by the method of Lowry et al. (20). Radioactivity was determined by liquid scintillation spectrometry after bleaching an aliquot of the supernate by boiling for 2 h in 15% perchloric acid and 8% H_2O_2. The latter step was necessary to prevent quenching. Results were expressed as counts per minute incorporated per milligram of protein.

RESULTS

Effect of carbohydrate administration on purine biosynthesis. Incorporation of radiolabeled glycine into hepatic purine was used to document that the dose of fructose administered in this animal model resulted in an accelerated rate of purine biosynthesis *de novo*. As shown by the data presented in Table I, approximately three times more radiolabeled carbon was incorporated into the hepatic adenine nucleotide pool of fructose-treated animals than either the glucose-treated or control animals. [^{14}C]glycine incorporation in the glucose-treated animals was not different from that observed in controls who received no carbohydrate. To control for potential changes in the specific activity of glycine in these different groups of animals, the incorporation of glycine into purine was also examined in relationship to glycine incorporation into protein. These data were expressed as follows: counts per minute per micromole of adenine/counts per minute per milligram of protein. The rate of glycine incorporation into purine relative to that into protein was approximately sixfold greater in the fructose- than glucose-treated or control animals. These results confirm that purine biosynthesis in liver is accelerated within 30 min following the intravenous injection of 2.5 mg of fructose per gram of body weight in the mouse.

TABLE I
Effect of Carbohydrate Infusion on the Incorporation of [^{14}C]Glycine into Hepatic Purine

	cpm/μmol adenine
Fructose-treated mice	
1	3,551
2	3,563
Glucose-treated mice	
1	1,030
2	1,269
Control	1,064

Mice were injected 30 min before death with 5 μCi of [^{14}C]glycine and the indicated carbohydrate. A control animal received no carbohydrate. The animals were killed and hepatic purine isolated as described in the methods. Results are presented as counts per minute incorporated per micromole of adenine.

Effect of carbohydrate administration on nucleotide content of liver. For these studies liver samples were obtained at 0, 3, and 30 min following the intravenous administration of 2.5 mg of carbohydrate/g of body wt. Fig. 1 illustrates the changes observed in total purine nucleotide content of liver (ATP + ADP + AMP + IMP + GTP + GDP + GMP) at each of these time points following carbohydrate injection. There was a significant decrease in total purine nucleotide content of liver in both the fructose and glucose-treated animals, but the magnitude of the change was greater in the animals that received fructose.

Results for individual nucleotides are presented in Table II. 3 and 30 min following carbohydrate infusion there was a significant reduction in the pools of ATP and GTP in both groups of animals, but the magnitude of the reduction was greater in the fructose than the glucose-treated animals. Since ATP is the single largest pool of purine nucleotide in the liver (52% of the total) and it exhibits the largest percentage reduction of any pool following fructose infusion, changes in the content of ATP largely account for the changes observed in the total pool of purine nucleotides (Fig. 1). However, as will be pointed out below, changes in the pools of other purine nucleotides, although less dramatic in quantitative terms, may be just as important for understanding the basis of the accelerated rate of purine biosynthesis that results from fructose infusion.

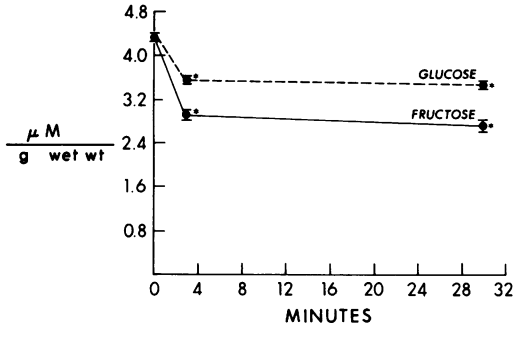

FIGURE 1 Purine nucleotide content of liver following carbohydrate infusion. The data plotted on the ordinate represent total purine nucleotides, i.e. AMP + ADP + ATP + IMP + GMP + GDP + GTP. Animals were killed at zero time (controls), 3 and 30 min following carbohydrate infusion. Glucose-treated animals are represented by - - - - - and fructose-treated animals by ——. The number of animals included in each data point are shown in Table II. The error bar represents ±1 SD from the mean. * Indicates a statistically significant ($P < 0.01$) difference between the value obtained at that time point and the control value, i.e., time zero.

In addition to these changes in the triphosphate pools, there was also a significant alteration in the hepatic content of ADP in the fructose-treated animals. As shown in Fig. 2, the ADP content was reduced by 32% at both 3 and 30 min following fructose administration, while ADP content of liver was not altered in the glucose-treated animals. GDP content was not significantly changed in either group of animals at either time point (Table II). The potential relevance of the decrease in ADP content of liver from fructose-treated animals will be addressed in the subsequent section of this paper dealing with PP-ribose-P metabolism.

There were also changes in the hepatic content of purine nucleoside monophosphates after carbohydrate administration. 3 min following carbohydrate infusion there was a significant increase in AMP and IMP in both groups of animals. 30 min following fructose administration the hepatic content of purine nucleoside monophosphates had returned to control values, whereas in the glucose-treated animals AMP and IMP concentrations remained elevated.

The combined effect of these changes in the different purine nucleotide pools on feedback inhibition of amidophosphoribosyltransferase is complex. Although this enzyme is sensitive to feedback inhibition by all purine nucleotides, the extent of inhibition is dependent on the state of phosphorylation of the nucleotide (14). For example, the amount of inhibition produced by triphosphates < diphosphates < monophosphates. Table III lists the relative potency of the different classes of purine nucleotides as inhibitors of purified mouse liver amidophosphoribosyltransferase. Using these data it is possible to generate one number that represents the combined inhibitory potential for all the purine nucleotides under each experimental conditon. This is accomplished by multiplying the concentration of each nucleotide (Table II) by its relative potency as an inhibitor (Table III). For example, 4.76 μmol of ATP produces the same amount of inhibition of amidophosphoribosyltransferase as 1.0 μmol of AMP. As illustrated in Fig. 3, there was a significant re-

TABLE II

Effect of Carbohydrate Administration on Purine Nucleotide Content of Mouse Liver

	Control	Fructose		Glucose	
Minutes after injection...	0	3	30	3	30
AMP	0.04±0.12	0.60±0.06	0.39±0.03	0.66±0.06	0.56±0.10
ADP	1.10±0.14	0.74±0.12	0.76±0.14	1.14±0.10	1.16±0.04
ATP	2.37±0.40	0.59±0.12	1.17±0.26	1.28±0.13	1.41±0.13
IMP	0.05±0.04	0.60±0.16	0.02±0.01	0.12±0.06	0.16±0.10
GMP	0.06±0.02	0.10±0.03	0.05±0.02	0.05±0.03	0.10±0.02
GDP	0.16±0.02	0.10±0.03	0.14±0.03	0.15±0.03	0.16±0.03
GTP	0.32±0.10	0.15±0.03	0.19±0.07	0.18±0.02	0.22±0.04
n	10	5	3	3	3

Mice were killed at 3 and 30 min following intravenous injection of 2.5 mg/g of fructose or glucose as described in the methods. Control animals were killed in the same manner but prior to injection of carbohydrate. Nucleotides were extracted from freeze-clamped liver and quantified by high performance liquid chromatography. Nucleotide content is expressed in micromoles of nucleotide per gram of wet liver weight. *n* = the number of animals in each group and the data are presented as the mean±1 SD for the group.

duction in the inhibitory potential for amidophosphoribosyltransferase in the fructose-treated animals, while there was no change in the inhibitory potential in the liver of glucose-treated animals.

Carbohydrate administration also led to a reduction in hepatic content of pyrimidine and pyridine nucleotides. Depletion and repletion of these nucleotide pools paralleled the changes in the purine nucleotide pools in both groups of animals. Since the hepatic content of pyrimidine and pyridine nucleotides is small in comparison to that of the purine nucleotides and these nucleotides are less potent as inhibitors of amidophosphoribosyltransferase (14), when the changes in hepatic content of these nucleotides were included in the computation of the inhibitory potential for amidophosphoribosyltransferase there was no significant alteration in the data presented in Fig. 3.

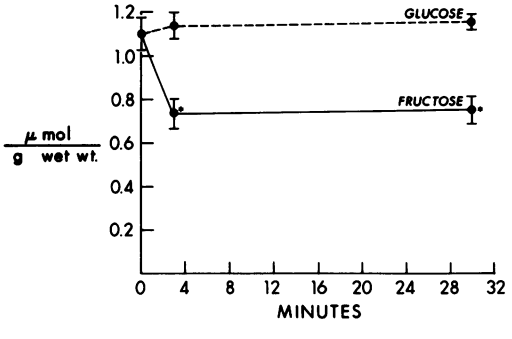

FIGURE 2 ADP content of liver after carbohydrate infusion. Symbols are the same as in Fig. 1.

Effect of carbohydrate administration on PP-ribose-P metabolism. While this as well as prior reports have documented that fructose administration leads to catabolism of purine nucleotides in liver (9), changes in hepatic content of PP-ribose-P following administration of fructose have not been described. As shown in Fig. 4, the hepatic content of PP-ribose-P increased 2.3-fold 30 min following intravenous infusion of fructose. There was no change in PP-ribose-P content of liver following glucose administration. As pointed out earlier, in the fructose but not the glucose-treated animals there was a significant reduction in the hepatic content of ADP, a nucleotide that is thought to be a physiologically important inhibitor of PP-ribose-P synthetase (25).

Effect of carbohydrate administration on amidophosphoribosyltransferase activity and conformation. Previous work has shown that human placental amidophosphoribosyltransferase undergoes an interconversion reaction between a small (133,000 dalton) and large (270,000 dalton) form of the enzyme following incubation with the ligands PP-ribose-P and purine nucleotides (13). These studies also suggested that the small form of amidophosphoribosyltransferase was catalytically active and the large form catalytically inactive. The data presented in Table IV illustrate that amidophosphoribosyltransferase from mouse liver also undergoes an interconversion reaction between a small and large form following incubation with PP-ribose-P and purine nucleotides. In the presence of PP-ribose-P the enzyme has an estimated mol wt of 127,000; following incubation with purine nucleotide

TABLE III

Relative Potency of Purine Nucleotides as Inhibitors of Mouse Liver Amidophosphoribosyltransferase

Nucleotide	Percentage inhibition	Relative potency as inhibitor
AMP	53	1.00
ADP	23	0.44
ATP	11	0.21
IMP	18	0.35
GMP	39	0.75
GDP	39	0.75
GTP	30	0.58

Mouse liver amidophosphoribosyltransferase was purified as described in Methods. The percentage inhibition of amidophosphoribosyltransferase activity produced by a 5-mM concentration of each purine nucleotide was determined in the regular enzyme assay, except the PP-ribose-P concentration was reduced to 1 mM and the $MgCl_2$ concentration increased to 10 mM. The center column gives the percentage inhibition of amidophosphoribosyltransferase activity produced by 5 mM nucleotide—the enzyme assayed without nucleotide produced 29.5 nM glutamate/h. The third column gives the relative potency of each nucleotide as an inhibitor of amidophosphoribosyltransferase. This was calculated as follows: AMP was found to be the most potent inhibitor, and it was assigned an arbitrary potency of 1. The relative potency of the other nucleotides as inhibitors of amidophosphoribosyltransferase was calculated by dividing the percentage inhibition produced by a given nucleotide by the percentage inhibition produced by AMP.

mouse liver amidophosphoribosyltransferase is converted to a protein with estimated mol wt of 292,000.

Since fructose administration in the mouse is associated with both a reduction in the hepatic content of purine nucleotides that are inhibitory for amidophosphoribosyltransferase and an increase in PP-ribose-P, liver extract from these animals was applied to a gel filtration column to determine whether the distribution

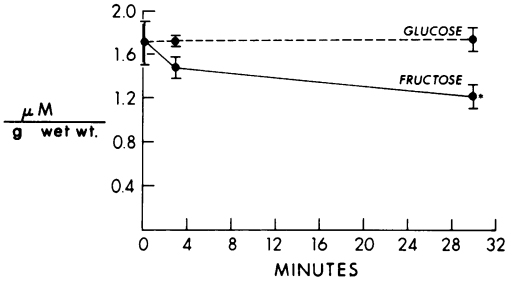

FIGURE 3 Inhibitory potential of hepatic nucleotides for amidophosphoribosyltransferase. Symbols are the same as in Fig. 1. Inhibitory potential was determined as follows: concentration of nucleotide (Table II) × potency as inhibitor of amidophosphoribosyltransferase (Table III). See text for further explanation of inhibitory potential.

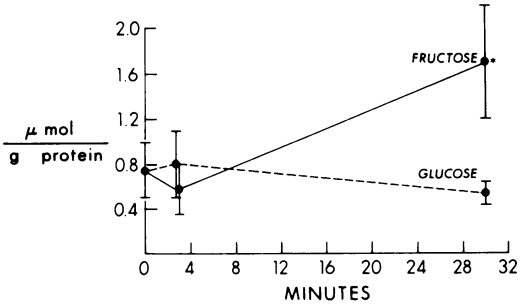

FIGURE 4 PP-ribose-P content of liver following carbohydrate infusion. n = 4 for the 0 time point; n = 5 for all other time points except 30 min following fructose infusion where n = 8. Symbols are the same as in Fig. 1.

of amidophosphoribosyltransferase between the small and large forms of the enzyme was affected by these changes. A typical gel filtration profile of amidophosphoribosyltransferase activity from extract of control liver is shown in Fig. 5A. For comparison, the profile of amidophosphoribosyltransferase activity from liver extract of a fructose-treated animal is shown in Fig. 5B. By calculating the area under the two activity peaks, such as shown in the profiles of Fig. 5, it is possible to determine the percentage of amidophosphoribosyltransferase activity in the small and large forms. A composite of the data obtained from liver extracts of animals killed at 0, 3, and 30 min following carbohydrate infusion is presented in Fig. 6. 30 min following fructose administration there was a significant increase in the percentage of amidophosphoribosyltransferase in the small form. At time zero there was 4.5±5.5% of amidophosphoribosyltransferase in the small form compared to 33.4±5.9 in the small form at 30

TABLE IV

Physical Properties of Mouse Liver Amidophosphoribosyltransferase

	$S_{20}W$ ($\times 10^{13}$S)	Stokes radius Å	Molecular weight
Incubation with PP-ribose-P	7.1±0.56 (n = 8)	42.0±1.87 (n = 4)	127,000
Incubation with purine nucleotide	10.5±0.61 (n = 10)	65.2±2.51 (n = 3)	292,000

Amidophosphoribosyltransferase was partially purified from mouse liver as described in Methods. Following incubation with 5 mM PP-ribose-P or 5 mM AMP the enzyme was applied to a sucrose density gradient or gel filtration column which also contained the same concentration of PP-ribose-P or AMP in buffer A. n = the number of determinations performed, and the data recorded in this table are the mean±1 SD of these determinations.

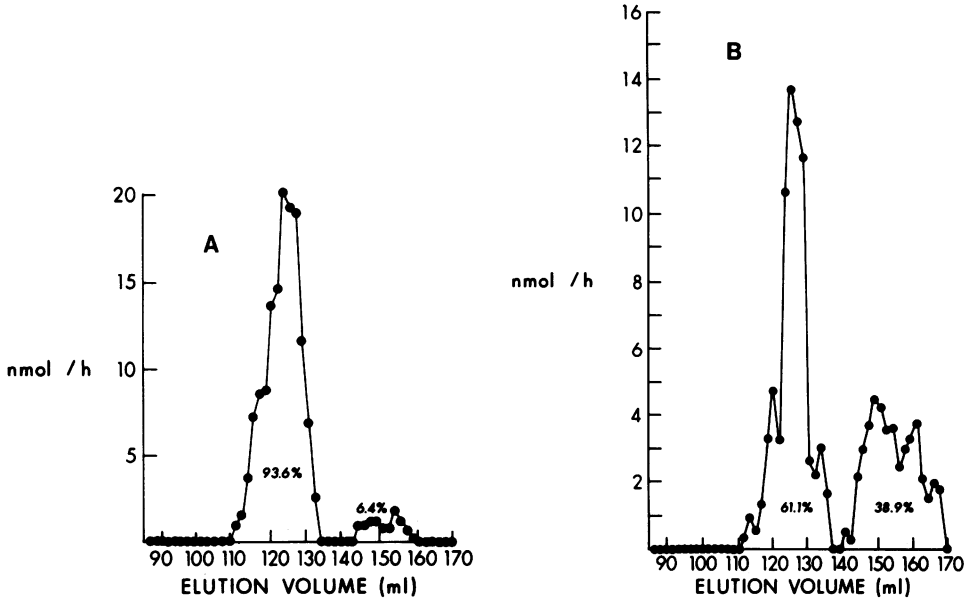

FIGURE 5 Profile of amidophosphoribosyltransferase activity from liver extract on an Ultrogel AcA-34 column. (A) Activity profile of liver extract from a control mouse. (B) Activity profile of liver extract from a mouse 30 min after fructose infusion. The numbers under each peak indicate the percentage of amidophosphoribosyltransferase activity in the large form, first peak, and in the small form, second peak.

min following fructose infusion ($P < 0.002$). Glucose administration did not alter the distribution of amidophosphoribosyltransferase between the small and large forms of the enzyme. Total amidophosphoribosyltransferase activity, i.e., the sum of the activities in the small and large forms of the enzyme, was not significantly different in liver extract from the fructose-treated animals when compared to either control or glucose-treated animals. Recovery of amidophosphoribosyltransferase activity from the gel filtration step was also comparable for the liver extract from the three groups of animals.

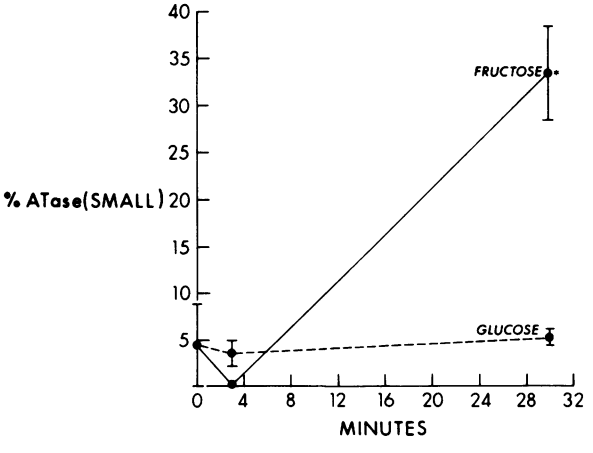

FIGURE 6 Percentage of amidophosphoribosyltransferase in the small form after carbohydrate infusion. $n = 3$ for each of the groups shown at 0, 3, and 30 min after carbohydrate infusion. Symbols are the same as in Fig. 1.

DISCUSSION

A number of studies have documented that fructose administration leads to the catabolism of hepatic adenine nucleotides, and this provides an adequate explanation for the abrupt onset of hyperuricemia observed following the administration of fructose. Catabolism of hepatic adenine nucleotides also accounts for the rapid increase in serum urate concentration observed in type I glycogen storage disease on fasting and following glucagon administration (8, 10). As a consequence of the catabolism of purine nucleotides there is an accelerated rate of purine biosynthesis *de novo* in both of these conditions (1–7). The present study was undertaken to provide an explanation for the increase in purine biosynthesis which results from the hepatic depletion of purine nucleotides. The model used for this study was validated by demonstrating that the dose of fructose administered resulted in both depletion of hepatic purine nucleotides and an increase in the rate of purine biosynthesis in the liver.

One mechanism by which a decrease in hepatic content of purine nucleotides could lead to an increase in purine synthesis is through a decrease in feedback inhibition of amidophosphoribosyltransferase, since previous studies have demonstrated that the activity of this enzyme is controlled by the end-products of the pathway (11–14). To determine the potential effect of

changes in nucleotide content of liver following carbohydrate infusion on amidophosphoribosyltransferase activity it is important to consider, not only the concentration of each nucleotide, but also the relative effectiveness of each class of nucleotide as an inhibitor of amidophosphoribosyltransferase (14). As pointed out above, a small increase in the concentration of purine nucleoside monophosphate could offset a large reduction in the concentration of nucleoside triphosphate with respect to inhibition of amidophosphoribosyltransferase, since the monophosphates are more potent inhibitors of this enzyme. In the glucose-treated animals the decrease in purine nucleoside triphosphates was offset by an increase in the concentration of nucleoside monophosphates, and although the total concentration of purine nucleotide was decreased, there was no change or even a slight increase in the inhibitory potential for amidophosphoribosyltransferase. In the fructose-treated animals there was a larger decrease in the concentration of purine nucleoside triphosphates and the increase in nucleoside monophosphates was of shorter duration. Thus, at the 30-min time point there was a significant decrease in the content of the inhibitory potential of nucleotides for amidophosphoribosyltransferase in the liver of fructose-treated animals. Therefore, the accelerated rate of purine biosynthesis observed in the animals that received fructose may be explained in part by a decrease in feedback inhibition of amidophosphoribosyltransferase resulting from depletion of hepatic purine nucleotides.

A second mechanism by which fructose administration could lead to an increase in the rate of purine synthesis is through an increase in PP-ribose-P content of liver, since many studies have documented a role for PP-ribose-P in the control of purine biosynthesis (11, 12, 17, 25). PP-ribose-P is an important ligand, just as purine nucleotides are, in the control of amidophosphoribosyltransferase activity (13, 14), and an increase in PP-ribose-P concentration can lead to an increase in amidophosphoribosyltransferase activity. Results of the present study demonstrate that fructose administration was associated with a 2.3-fold increase in the hepatic content of PP-ribose-P. There was no change in the hepatic content of PP-ribose-P in the glucose-treated animals. Although there may be other mechanisms that contribute to the increase in PP-ribose-P content of liver following fructose infusion, one explanation found in the data presented here suggests that the increase in PP-ribose-P may be a direct consequence of purine nucleotide depletion. In the fructose-treated animals there was a 33% reduction in hepatic content of ADP, a potent inhibitor and important ligand in the control of PP-ribose-P synthetase activity (25). In the glucose-treated animals there was no change in the hepatic content of ADP. Thus, a second explanation for the increased rate of purine biosynthesis that results from fructose administration may be as follows: decrease in ADP content of the liver → increase in PP-ribose-P synthetase activity → increase in PP-ribose-P content of liver → activation of amidophosphoribosyltransferase → increase in purine synthesis.

The basis for the accelerated rate of purine biosynthesis that results from nucleotide depletion need not be limited to only one of the above mechanisms. Rather than being exclusive, these two mechanisms may be operative simultaneously and exert a synergistic effect on purine biosynthesis. The combined effect of changes in the concentration of nucleotides and PP-ribose-P on purine synthesis can be understood at the enzymatic level by examining the response of amidophosphoribosyltransferase to a decrease in the concentration of inhibitory nucleotides and an increase in the concentration of PP-ribose-P. In mechanistic terms purine nucleotides behave like competitive inhibitors of amidophosphoribosyltransferase with respect to PP-ribose-P (14). The conversion of the large inactive to the small active form of amidophosphoribosyltransferase is determined by the relative concentrations of PP-ribose-P and purine nucleotides (13). Thus, in the liver of fructose-treated mice both the decrease in concentration of inhibitory nucleotides and increase in concentration of PP-ribose-P would be expected to lead to a shift of the large to the small form of amidophosphoribosyltransferase. This prediction was realized in the demonstration of the conversion of 28% of amidophosphoribosyltransferase from the large to small form of the enzyme. Since amidophosphoribosyltransferase catalyzes the first and potential rate-limiting reaction in this biosynthetic pathway (11–14), conversion of the large inactive to the small active form of amidophosphoribosyltransferase provides an explanation at the enzymatic level for the accelerated rate of purine biosynthesis observed after nucleotide depletion.

The sequence of events depicted by the model in Fig. 7 summarizes the studies presented here regarding the basis for the increase in purine biosynthesis that results from nucleotide depletion. As a consequence of purine nucleotide depletion, such as observed after fructose administration or during fasting in type I glycogen storage disease, there is a decrease in feedback inhibition of amidophosphoribosyltransferase and PP-ribose-P synthetase. The increase in PP-ribose-P synthetase activity leads to an increase in PP-ribose-P production and concentration. The combination of decreased feedback inhibition of amidophosphoribosyltransferase by nucleotides and increased concentration of PP-ribose-P leads to a shift from the large inactive to the small active form of the enzyme. The resultant increase in amidophosphoribosyltransferase activity leads to an increase in the rate of purine nucleotide synthesis, thereby providing a mechanism by which the end-products of the pathway control the activity of the rate-limiting reaction for nucleotide synthesis.

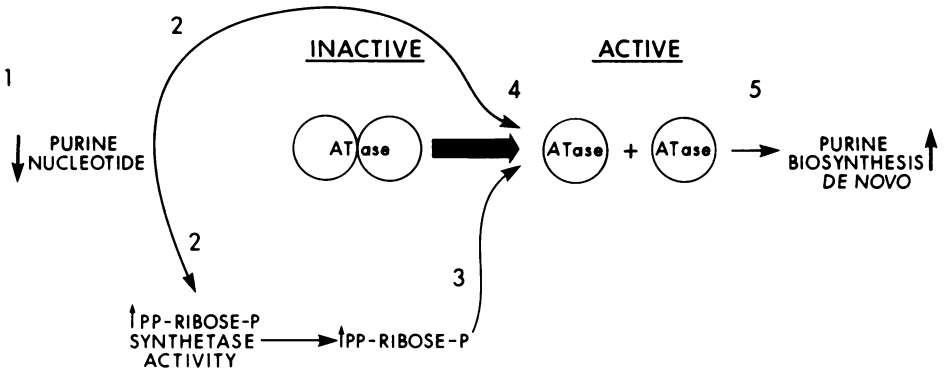

FIGURE 7 Model for the control of purine biosynthesis by purine nucleotides. Numbers indicate temporal sequence of events. ATase is abbreviation for amidophosphoribosyltransferase.

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