Liver Adenine Nucleotides: Fructose-Induced Depletion and Its Effect on Protein Synthesis

Abstract. *Intravenous administration of* D-*fructose to rats rapidly depletes liver adenosine triphosphate and inorganic phosphate; marked elevations of uric acid and allantoin in plasma follow. Concomitantly the incorporation of* DL-*leucine-1-*^{14}*C into liver protein is almost completely inhibited.*

Intravenous injection of D-fructose in normal humans significantly increases uric acid in plasma, with concomitant increase in urinary excretion of urate (*1*). The rapid development of the hyperuricemia, to a maximum 15 minutes after the injection, is difficult to explain by acute increase in *de novo* synthesis of purine, but increased catabolism of preformed purine nucleotides presents a plausible mechanism for the phenomenon. We have studied the effect of fructose on hepatic levels of adenine nucleotides in the rat, and attempted to evaluate some of the metabolic consequences.

We used young Sprague-Dawley rats weighing 150 to 200 g and fed on a conventional laboratory diet. Fructose or a control compound (1 mmole of each) was injected into the inferior vena cava exposed to laparotomy under thiopentone sodium anesthesia.

First we studied the effect of fructose on the levels in plasma of uric acid and allantoin (which is the end product of purine catabolism in the rat). The needle used for injection of fructose was filled with heparin and left *in situ*, and blood samples were drawn at the indicated times (Fig. 1); uric acid was determined with the aid of uricase (*2*), and allantoin was measured colorimetrically (*3*). Injection of fructose was followed by highly significant elevations in the levels of uric acid and allantoin in plasma, both of which increased about fourfold within 15 minutes (see Fig. 1). Thus the rat's response to fructose qualitatively resembled man's (*1*).

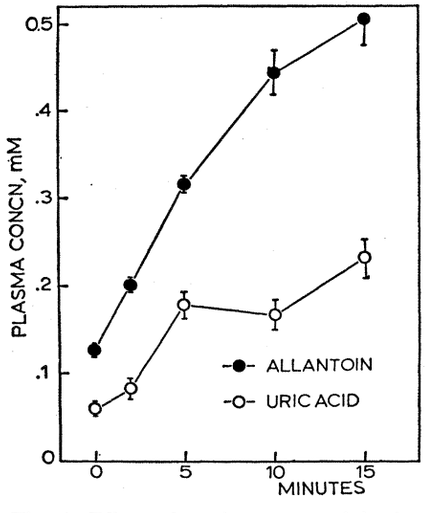

Fig. 1. Effect of an intravenous injection of 1 mmole of fructose on the plasma concentrations of allantoin and uric acid. Each point is the mean, ± S.E. of the mean, of four measurements at each of the indicated times after the injection.

In order to determine the source of the increased amounts in plasma of purine-degradation products, we studied the effects of an identical dose of fructose on the hepatic levels of adenine nucleotides and inorganic phosphate. At intervals after the injection of fructose (or of equimolar amounts of glucose, galactose, ribose, or sorbitol), samples of liver, taken with metal tongs precooled in liquid nitrogen, were homogenized in 0.6*M* perchloric acid and centrifuged in the cold. The supernatants were used for determination of the adenine nucleotides (*4*) and inorganic phosphate (*5*).

One minute after the injection, the hepatic content of adenosine triphosphate (ATP) was significantly depressed; after 5 minutes it reached its nadir at 40 percent of the initial level (Fig. 2A). Smaller and more transient increments in adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were concomitant, the net effect being depression of total adenine nucleotides to approximately 53 percent of the control level at 5 minutes (Fig. 2B). Subsequently a slow increase in ATP and progressive depressions in ADP and AMP occurred, but at 30 minutes the content of ATP and total adenine nucleotides was still significantly lower than in the controls.

In comparison with these changes, the level of inorganic phosphate in liver tissue fell even more rapidly and extensively, reaching half the initial value within 1 minute and reaching one-third within 5 minutes of the injection (Fig. 2B). However, the rebound also was rapid, the values at 20 and 30 minutes even exceeding the control levels.

In a series of control experiments, with either glucose, galactose, ribose, or sorbitol injected instead of fructose, only sorbitol significantly depressed hepatic ATP ($P < .02$) and total adenine nucleotides ($P < .05$); its effect was, however, quantitatively much smaller than that of fructose. The other control compounds did not significantly alter the nucleotide levels.

Because ATP plays an important role in protein synthesis, we studied the effect of fructose-induced depletion of ATP on the incorporation of DL-leucine-1-^{14}C into liver protein. Two microcuries of the label (specific activity, 55.2 mc/mmole) was injected at various times relative to an injection of 1 mmole of fructose, and a sample of liver was taken in each instance (by the rapid-freezing technique described) 3 minutes after the leucine injection. The protein in the sample was precipitated and washed (*6*), and its radioactivity was counted (*7*). The protein contents of the processed samples were measured (*8*), the results being expressed as incorporated counts per minute per gram of protein.

When leucine was injected 2 minutes after an intravenous dose of fructose, the amount of radioactivity incorporated into liver protein was depressed to less than 10 percent of the control value (Fig. 3), the fall indicating significantly decreased synthesis of protein. With an interval of 12 minutes between the two injections, the amount of label incorporated was still less than half the control value, whereas, with

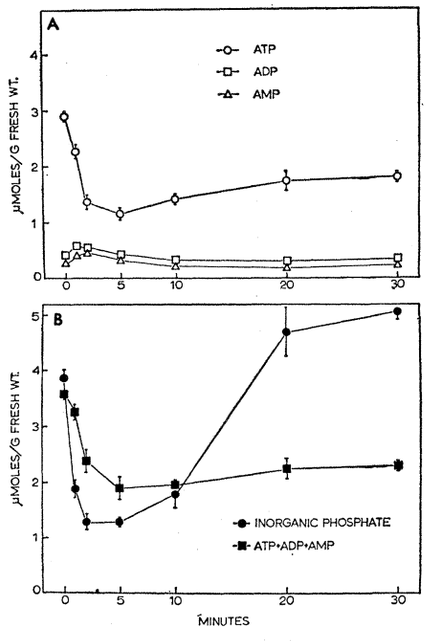

Fig. 2. Content of the adenine nucleotides and of inorganic phosphate in rat liver after an intravenous injection of 1 mmole of fructose. The liver was sampled at the indicated times after the injection. Each point is the mean, ± S.E. of the mean, of five to nine separate experiments. (A) Levels of ATP, ADP, and AMP, given separately. (B) Levels of inorganic phosphate and total adenine nucleotides.

an interval of 27 minutes, control values were again approached. Comparison of the time-courses of the changes, in ATP levels and protein synthesis in the liver (expressed in terms of incorporation of radioactivity from DL-leucine-1-^{14}C), indicates good correlation between these two variables (Figs. 2A and 3).

In the regulation of intracellular purine nucleotide levels, adenylate deaminase seems to have an important function (*9*). This view is supported by the known regulatory properties of the enzyme—mainly stimulation by ATP and inhibition by guanosine triphosphate (*10*) as well as by inorganic phosphate, which is readily demonstrable at physiological concentrations (*11*).

The metabolism of fructose, which is localized almost entirely in the liver (*12*), involves a rapid initial phosphorylation [estimated to occur three times as rapidly as that of glucose (*13*)] followed by the aldolase reaction which is considered to be rate-limiting (*14*). Therefore the administration of fructose would be expected to result in an initial decrease in content of ATP in the liver, with corresponding increase in ADP and AMP, accumulation of fructose-1-phosphate [which has been demonstrated (*15*)], and decrease in inorganic phosphate due to its "sequestration" in the form of fructose-1-phosphate (see Fig. 2B).

Contrary to these expectations, the decrease in ATP is not accounted for by corresponding increase in ADP and AMP (Fig. 2A), but rather by increased production of uric acid and allantoin, with total adenine nucleotides diminishing as the result. This phenomenon can be explained by the properties of adenylate deaminase: as the intracellular level of inorganic phosphate falls, this enzyme is strongly activated, and a transient increase in substrate (AMP) concentration evidently favors the accelerated breakdown of the adenine nucleotides by this route. However, as shown in Fig. 2, A and B, the fructose effect is brief, the suggestion being rapid function of counterregulatory mechanisms that prevent further depletion of the adenine nucleotide pool. Decreasing levels of substrate (AMP) and activator (ATP) of adenylate deaminase probably contribute to deceleration of the rate of deamination of AMP.

Certain questions associated with the metabolism of fructose deserve consideration in the light of our findings.

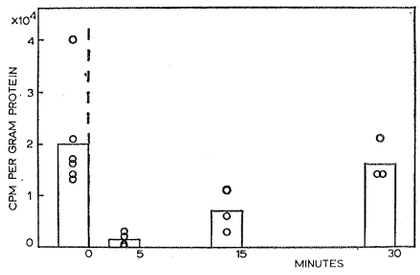

Fig. 3. Specific radioactivity of liver protein 3 minutes after an intravenous injection of 2 μc of DL-leucine-1-^{14}C, alone or at various times after an injection of 1 mmole of fructose. The left sides of the columns represent the times of injection of leucine; the right sides, the times of sampling. The temporal relation to the fructose injection is shown on the horizontal axis. The lengths of the columns represent the means for the experiments indicated by the circles. The results are expressed as counts per minute (*CPM*) incorporated per gram of liver protein.

The marked depression of synthesis of protein, concomitantly with the low levels of ATP, is analogous to the sequence of events after the administration of ethionine (*16*), although the time courses of the two phenomena are quite different. Although the utilization of the intracellular ATP for fructose phosphorylation, in preference to protein synthesis, is evidence against the concept of compartmentation of extramitochondrial ATP, definite conclusions seem to be unwarranted.

Another interesting result of administration of fructose is the transient depression of glucose levels in the blood, which is most marked in patients having hereditary intolerance to fructose (*17*) and in newborn infants (*18*), but occurs even in normal adults with larger doses (*19*). In patients having hereditary intolerance to fructose and in newborn infants the depression can be prevented by administration of galactose but not of glucagon (*20*). On this basis, some metabolite of fructose has been postulated to interfere with glycogenolysis (*21*), although the exact mechanism has not been demonstrated. However, as ATP is essential for conversion of the inactive phosphorylase-b to the active a-form in the liver (*22*), failure of this conversion, due to the fructose-induced depletion of ATP, offers a possible explanation of the inadequate rate of glycogenolysis. Here again an analogy can be drawn to results of studies of the effects of ethionine in the liver, demonstrating correlation between the depression of levels of ATP and diminution of phosphorylase activity (*23*).

The marked elevation of levels of uric acid in plasma after fructose is administered indicates that the purine content is not the only important factor in the diet of patients with gout. Although the effects of chronic administration of fructose on adenine nucleotide catabolism have not yet been studied, one may conceive that repetitive stimuli in the form of high intake of fructose place extra demands on purine synthesis as well. Therefore dietary habits must be regarded as potential factors when one attempts to explain the increased incidence of hyperuricemia and gout in the higher social groups (*24*).

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