Biochemical Pharmacology, Vol. 18, pp. 2615–2624. Pergamon Press. 1969. Printed in Great Britain

DEPLETION OF LIVER ADENINE NUCLEOTIDES INDUCED BY D-FRUCTOSE

DOSE-DEPENDENCE AND SPECIFICITY OF THE FRUCTOSE EFFECT

KARI O. RAIVIO, MARTTI P. KEKOMÄKI and PEKKA H. MÄENPÄÄ

Children's Hospital and Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

(*Received* 10 *February* 1969; *accepted* 30 *May* 1969)

Abstract—The effect of D-fructose on adenine nucleotide metabolism was studied in rats *in vivo* by analyzing liver metabolite levels after intravenous fructose injections, and *in vitro* by measuring allantoin production by the isolated perfused liver after the addition of fructose to the perfusion medium.

Significant depression of liver ATP and inorganic phosphate levels was demonstrated after fructose administration. Minimum effective dose was 0·25 m-mole, and maximum depression was observed after 5 min. Analogous, but quantitatively less impressive alterations were produced by L-sorbose and D-sorbitol, whereas D-glucose, D-galactose, D-mannose, D-ribose and 2-deoxy-D-glucose were without effect.

In the perfusion studies, marked increase in allantoin production was observed after the addition of D-fructose to the perfusion medium. The minimum effective dose was 0·25 m-mole, corresponding to a mean initial concentration of 4·5 mM in the medium. Allopurinol prevented both the basal allantoin production and the fructose-induced increase. D-galactose, D-ribose, D-sorbitol, sodium-DL-lactate or ethanol did not influence the basal rate of allantoin release.

The loss of liver adenine nucleotides in the *in vivo* experiments was of a similar order of magnitude as the amount of allantoin produced in the perfusion experiments, when calculated per total liver after equal doses of fructose. Therefore, accelerated degradation of preformed purines, rather than increased *de novo* synthesis, appears to be the main mechanism of fructose-induced increase in uric acid and allantoin production.

INTRAVENOUS injection of D-fructose into human individuals is followed by a rapid increase in serum uric acid levels.[1] We have previously demonstrated that an analogous response can be elicited in rats, and that concomitantly liver adenine nucleotide, especially ATP, levels are markedly depressed.[2] Furthermore, inhibition of incorporation of radioactivity from uniformly labelled leucine-^{14}C into liver proteins was documented and postulated to be due to the adenine nucleotide depletion.[2]

In order to confirm and extend these findings, experiments were performed with the aims of establishing the specificity of the fructose effect, investigating the dose–response relationship, and elucidating the mechanisms underlying the adenine nucleotide depletion.

MATERIALS AND METHODS

Female rats of the Sprague–Dawley strain were used. They were fed on a standard laboratory diet *ad libitum* prior to the experiments, and they weighed 150–200 g.

Two lines of investigation were followed. In a series of *in vivo* experiments, D-fructose was injected in a 9% aqueous solution into the inferior vena cava, exposed at laparotomy under thiopentone anaesthesia. Liver samples were taken with metal tongs precooled in liquid nitrogen. After rapid weighing, the frozen samples were homogenized for 5 min in 10 vol. of cold 0·6 N perchloric acid with a teflon pestle in a glass homogenization vessel. The homogenates were centrifuged in the cold at 5000 *g* for 5 min, and the supernatants were analyzed within a few hours of the removal of the sample. ATP, ADP, and AMP were assayed with the NAD-coupled methods of Adam,[3] lactate[4] and pyruvate[5] with enzymatic methods, and inorganic phosphate (P_i) with a modification of the procedure of Fiske and Subbarow.[6]

These techniques of injection, sampling, and analysis were employed in studies on the time-course of the metabolite changes after the administration of 1·0 m-mole of D-fructose, in which liver samples were taken 1, 2, 5, 10, 20, or 30 min after injection. Furthermore, the effect of varying doses of D-fructose on liver ATP levels was investigated by injecting 0·1, 0·25, 0·5, or 2·0 m-moles and sampling 5 min thereafter. Hepatic ATP and P_i contents were also measured in samples taken 5 min after an analogous injection of 1·0 m-mole of D-glucose, D-galactose, L-sorbose, D-mannose, 2-deoxy-D-glucose, D-ribose, or D-sorbitol. In a number of animals, the effects of D-fructose, L-sorbose, or D-mannose on kidney or heart ATP and P_i levels were studied with similar methods.

In a separate series of experiments, isolated rat liver was perfused according to a previously described technique.[7] The initial volume of the recirculating Krebs–Ringer bicarbonate solution, containing glucose in a concentration of 11 mM, was 60 ml. In some experiments, allopurinol* (4-hydroxypyrazolo (3, 4-*d*)-pyrimidine) was present in a concentration of 10 mg/100 ml. Test compounds (D-fructose, D-galactose, D-ribose, D-sorbitol, sodium-DL-lactate, or ethanol) were added to the medium after an equilibration period of 60 min, and the total duration of perfusion was 120 min. Samples (3 ml) of the medium were drawn 30, 45, 60, 75, 90 and 120 min after the beginning of each experiment, except in the control perfusions, in which the samples were taken at 5, 15, 30, 45, 60, 90 and 120 min. Aliquots of the samples were precipitated with perchloric acid for the determination of glucose[8] and lactate,[9] and of fructose,[10] when this or sorbitol had been added. The rest of the samples were kept frozen until the allantoin[11] and uric acid[12] analyses, which were completed within 48 hr. After perfusion the liver was cleaned of extra tissue and homogenized in distilled water. The homogenate was used for the determination of total liver protein.[13]

Results are expressed as means ± S.E.M., with the exception of certain control experiments performed only in duplicate, in which cases the averages of the two sets of results are given. The statistical significance of differences between means was established by the Student *t*-test. Data on liver metabolite levels *in vivo* have been calculated per gram fresh weight, but because of the potential errors in weighing the perfused livers their protein content has been used as the basis for comparison.

RESULTS

Time-course of liver metabolite changes induced by 1 *m-mole of* D-*fructose* in vivo (Table 1)

The depression of ATP and total adenine nucleotides was already apparent at 1 min after intravenous fructose injection, and was maximal at 5 min, whereafter

*A gift of OY Medikalis AB.

TABLE 1. CONTENT OF ADENINE NUCLEOTIDES, LACTATE, PYRUVATE AND INORGANIC PHOSPHATE IN LIVER AFTER AN INTRAVENOUS INJECTION OF 1 m-mole OF D-FRUCTOSE

	Control	Fructose					
Time after injection (min)		1	2	5	10	20	30
No. of observations		6	5	5	6	6	4
Content of metabolites (μmoles/g fresh weight)							
ATP	2·90 ± 0·09	2·27 ± 0·12†	1·37 ± 0·13‡	1·16 ± 0·12‡	1·41 ± 0·08‡	1·75 ± 0·17‡	1·80 ± 0·08‡
ADP	0·41 ± 0·04	0·59 ± 0·04†	0·55 ± 0·03*	0·42 ± 0·03	0·34 ± 0·02	0·31 ± 0·03	0·33 ± 0·02
AMP	0·26 ± 0·03	0·40 ± 0·02†	0·46 ± 0·05†	0·33 ± 0·04	0·21 ± 0·02	0·19 ± 0·01	0·13 ± 0·01
ATP + ADP + AMP	3·57 ± 0·08	3·25 ± 0·13	2·38 ± 0·19‡	1·90 ± 0·07‡	1·96 ± 0·07‡	2·24 ± 0·19‡	2·25 ± 0·09‡
Inorganic phosphate	3·86 ± 0·16	1·89 ± 0·16‡	1·28 ± 0·14‡	1·29 ± 0·07‡	1·79 ± 0·26‡	4·69 ± 0·44	5·42 ± 0·09‡
Lactate	2·39 ± 0·14	2·53 ± 0·24	2·77 ± 0·27	3·10 ± 0·24*	2·85 ± 0·22	3·48 ± 0·48*	1·87 ± 0·16*
Pyruvate	0·24 ± 0·01	0·24 ± 0·03	0·18 ± 0·01‡	0·15 ± 0·01‡	0·28 ± 0·03	0·20 ± 0·01†	0·22 ± 0·03
Lactate + pyruvate	2·62 ± 0·14	2·76 ± 0·26	2·96 ± 0·28	3·25 ± 0·24*	3·13 ± 0·26	3·69 ± 0·49*	2·09 ± 0·18*
Ratio lactate/pyruvate	10·1	10·6	15·3	20·4	10·2	17·1	8·6

Figures are means ± S.E.M. of the number of experiments given. * = $P<0.05$, † = $P<0.01$, and ‡ = $P<0.001$.

slow elevation became evident. However, at 30 min the levels were still significantly lower than in livers of untreated rats. The changes in ADP and AMP contents were less conspicuous and after transient elevations during the first 2 min the levels remained at or below control values.

An immediate and pronounced fall in liver P_i was observed, with a nadir at 2 to 5 min. However, the ensuing rapid rebound resulted in values exceeding control levels at 20 and 30 min.

The fructose-induced changes in liver lactate and pyruvate levels were less marked than those described above. At 5 min, a moderate rise in lactate and a significant fall in pyruvate resulted in doubling of the lactate/pyruvate ratio and a moderate increase in the total amount of lactate plus pyruvate. Both compounds attained their control levels by 30 min.

TABLE 2. EFFECT OF VARIOUS DOSES OF FRUCTOSE ON LIVER ATP CONTENT

Dose (m-moles)	No. of experiments	Liver ATP (μmoles/g fresh weight) mean ± S.E.M.
Control	9	2·90 ± 0·09
0·10	5	3·44 ± 0·08
0·25	5	2·38 ± 0·10†
0·50	5	1·97 ± 0·11‡
1·00	5	1·16 ± 0·12‡
2·00	5	1·14 ± 0·08‡

Liver samples were taken 5 min after an intravenous injection of fructose. Asterisks etc. as in Table 1.

Effect of varying doses of fructose on liver ATP content in vivo (Table 2)

An i.v. injection of 0·25 m-mole of fructose brought about a significant decrease in liver ATP content, and with the dose of 0·5 m-mole the effect was highly significant. Maximum depression was obtained with 1·0 m-mole, and doubling of this dose caused no further ATP depletion.

Effect of related monosaccharides on liver ATP and P_i content in vivo (Table 3)

Comparison of the effects of equivalent doses of the other test compounds with those of fructose indicated that only L-sorbose caused a significant fall in liver ATP content, and concomitantly the largest depression of P_i, with the exception of fructose. With D-sorbitol, a moderate decrease in ATP was noted, but the fall in P_i induced by D-glucose or D-mannose was unaccompanied by changes in ATP levels.

Effect of fructose and related monosaccharides on kidney and heart ATP and P_i levels in vivo (Table 4)

Moderate depression of kidney ATP content was induced by fructose, but the P_i data are inconclusive because of one grossly high value. None of the monosaccharides studied had any effect on the metabolite levels in heart tissue.

Production of allantoin by the perfused liver (Fig. 1)

In preliminary experiments it was established that, under the conditions described,

TABLE 3. LIVER ATP AND INORGANIC PHOSPHATE CONTENT AFTER AN INTRAVENOUS INJECTION OF 1 m-mole OF VARIOUS MONOSACCHARIDES AND SORBITOL

Compound injected	No. of experiments	ATP (μmoles/g fresh weight) mean ± S.E.M.	Inorganic phosphate (μmoles/g fresh weight) mean ± S.E.M.
None	9	2·90 ± 0·09	3·86 ± 0·16
D-fructose	5	1·16 ± 0·12‡	1·29 ± 0·07‡
D-glucose	5	3·25 ± 0·14	2·84 ± 0·16†
D-galactose	4	3·30 ± 0·16	3·10 ± 0·12
L-sorbose	4	2·17 ± 0·07‡	1·47 ± 0·07‡
D-mannose	4	2·92 ± 0·02	2·74 ± 0·10‡
2-deoxy-D-glucose	5	2·85 ± 0·10	3·38 ± 0·51
D-ribose	4	3·09 ± 0·24	—
D-sorbitol	4	2·26 ± 0·20*	—

Liver samples were taken 5 min after the injection. Asterisks etc. as in Table 1.

TABLE 4. ATP AND INORGANIC PHOSPHATE LEVELS IN RAT KIDNEY AND HEART 5 min AFTER AN INTRAVENOUS INJECTION OF 1 m-mole OF FRUCTOSE OR RELATED MONOSACCHARIDES

Compound injected		Metabolite levels (μmoles/g fresh weight) Kidney ATP	Kidney P_i	Heart ATP	Heart P_i
None	mean	2·13 (3)	2·39 (2)	4·54 (5)	7·79 (5)
	range	1·83–2·29	2·37–2·40	4·41–4·67	7·35–8·12
Fructose	mean	1·42 (3)*	2·80 (3)	4·37 (3)	8·51 (3)
	range	1·33–1·49	1·81–4·21	4·08–4·70	8·39–8·66
Sorbose	mean			4·69 (4)	8·72 (4)
	range			4·21–5·07	8·30–9·10
Mannose	mean			4·64 (4)	8·93(4)
	range			4·10–5·37	7·90–10·00

Number of experiments in parentheses. Asterisks etc. as in Table 1.

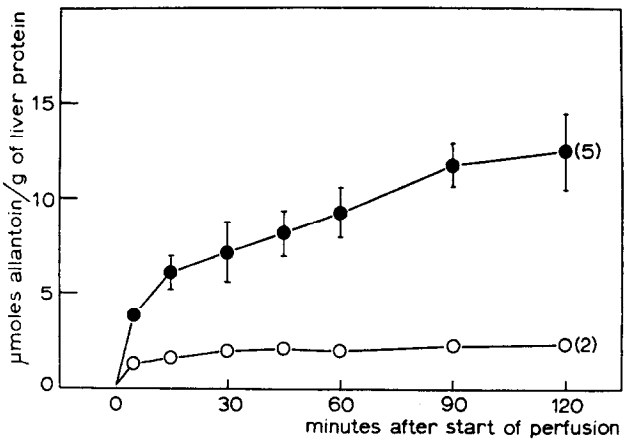

FIG. 1. Production of allantoin by isolated rat liver perfused with standard medium (closed circles) or with medium containing allopurinol (open circles). Number of experiments in parentheses.

essentially no uric acid appeared in the perfusion medium before or after the addition of 0·5 m-mole of fructose. Subsequently, uric acid was not measured in the perfusion samples.

Five control perfusions without additions were performed. Allantoin was continuously released from the liver, the mean amount accumulating in the medium in 2 hr being 12·5 μmoles/g liver protein. After the first 15 min of rapid production, during which approximately half the total amount was released, the rate of production was almost linear. Calculation on the basis of this rate gives an estimated daily production of allantoin by the whole liver of 184 μmoles or 29 mg (mean total liver protein was 1·2 g). This value is in agreement with the daily allantoin excretion of 20 mg, measured in intact rats.[14]

The presence of allopurinol in a concentration of 10 mg/100 ml in the medium depressed the amount of allantoin produced in 2 hr to 2·4 μmoles/g liver protein. Thus, the concentration of allopurinol used seems to inhibit the xanthine oxidase of the liver effectively.[15]

As the experimental animals had been fed *ad libitum*, the livers contained variable amounts of glycogen. Considerable amounts of glucose and lactate appeared in the medium during each perfusion, but the variation between experiments was large and bore no relation to the allantoin production. Allopurinol had no detectable effect on the release of glucose or lactate.

Effect of varying doses of fructose on allantoin production by the perfused liver (Fig. 2)

Addition of fructose to the perfusion medium after 60 min of equilibration elevated the basal allantoin production markedly. The minimum effective amount was 0·25 m-mole, and increasing doses of fructose brought about increased responses. With

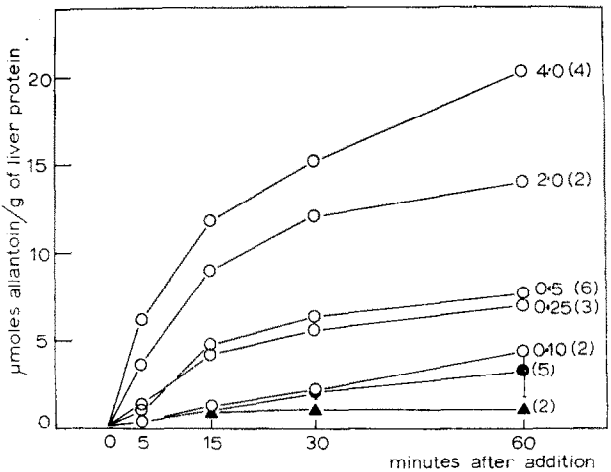

FIG. 2. Effect of fructose on the production of allantoin by isolated perfused rat liver. Varying doses of fructose were added to the medium at zero time (after 60 min of equilibration), and the increments above the amount produced by that time are indicated (open circles). In two experiments with 0·5 m-mole of fructose, the medium contained allopurinol (closed triangles). For comparison, values of control perfusions without additions are given (closed circles). Number of experiments in parentheses.

2·0 and 4·0 m-moles highly significant increases in allantoin production were already observed 5 min after addition, but with 0·25 and 0·5 m-mole only after 15 min. One hr after the addition of fructose, only the results with 2·0 and 4·0 m-moles differed significantly from controls.

In two experiments, the perfusion medium contained 10 mg/100 ml of allopurinol, and 0·5 m-mole of fructose was added. Only a negligible change in the low basal allantoin release was observed.

Fructose added in amounts of 0·1, 0·25, or 0·5 m-mole disappeared rapidly and practically linearly from the medium, with a mean half-time of 12 min (range 9–16 min). The final conecntration was less than 0·5 mM in each of these experiments, whereas elimination of 2·0 or 4·0 m-moles was far from complete in 60 min, the mean final concentrations being 15 and 50 mM, respectively.

Addition of fructose caused a rapid elevation of the lactate levels of the medium, which reached a maximum after 30 min and subsequently decreased. The concentration of glucose in the medium also increased considerably after fructose addition, with a maximum at 60 min. However, the quantitative changes in glucose and lactate were very variable, presumably because of the unknown contribution of hepatic glycogen stores.

Two perfusions were done with livers from rats starved for 48 hr. After the first hour of perfusion the average amounts of glucose and lactate accumulated in the medium were 50 and 10 μmoles/g liver protein, respectively, i.e. approximately 1/7 and 1/30, respectively, of those observed in experiments with fed rats. The addition of 0·5 m-mole of fructose caused a 6-fold increase in glucose and 10-fold in lactate of the medium after 60 min, but the alteration in the release of allantoin did not differ from that caused by a similar amount of fructose in the livers of fed animals.

Effect of other test compounds on allantoin production by the perfused liver (Table 5)

In order to confirm the specificity of the effect of fructose, control perfusions were performed by adding to the medium, instead of fructose, 0·5 m-mole of D-galactose,

TABLE 5. EFFECTS OF GALACTOSE, RIBOSE, SORBITOL, LACTATE AND ETHANOL ON THE PRODUCTION OF ALLANTOIN BY THE PERFUSED RAT LIVER

Compound added		Increment in the amount of allantoin (μmoles/g liver protein) Time (min) 5	15	30	60
None				2·1 ± 0·4	3·3 ± 1·5
Galactose	(0·5 m-mole)	0·7	0·8	2·7	4·5
Ribose	(0·5 m-mole)	1·2	2·5	3·9	6·7
Sorbitol	(0·5 m-mole)	0·4 ± 0·1	2·0 ± 0·5	3·8 ± 1·8	5·3 ± 2·1
Lactate	(1·0 m-mole)	1·2	1·9	3·3	6·5
Ethanol	(1·0 m-mole)	0·1	0·7	2·1	4·5

The test compounds were added to the perfusion medium at 60 min and samples were taken at the designated intervals. The figures are increments over the amount of allantoin produced during the equilibration period. Means ± S.E.M. of five experiments without additions and six with addition of sorbitol are given. The other values are averages of the results of two experiments.

D-ribose, or D-sorbitol. The importance of fructose-induced hyperlactatemia[1] in the causation of allantoin release was studied by adding 1·0 m-mole of sodium-DL-lactate to the perfusion medium. Furthermore, the role of the shift in cytoplasmic redox potential, indicated by the increased lactate/pyruvate-ratio after fructose administration, was investigated by adding 1·0 m-mole of ethanol, which is known to alter the redox state in the same direction.[16] As shown in Table 5, none if these test compounds caused significant changes in the production of allantoin in comparison to the perfusions without additions. After the addition of sorbitol, small amounts of fructose appeared in the medium, but the concentration was less than 1 mM in each case and returned to practically zero by 60 min.

DISCUSSION

Although the changes in liver adenine nucleotide levels after fructose administration were very pronounced, estimation of the actual amounts degraded is difficult because of the dynamic state existing *in vivo*. Therefore, the observed fall in total adenine nucleotide content 5 min after an injection of 0·5 m-mole of fructose, 0·93 μmole/g fresh weight (Table 2) or approximately 4·7 μmoles/total liver (mean liver weight of the experimental animals was 5 g), underestimates the true degraded amount by the unknown quantity resynthesized during the same period. A part of the deficit in adenine nucleotides appears as allantoin, the rest being accounted for by intermediates of purine catabolism. Nevertheless, the amount of allantoin released into the perfusion medium in 60 min after the addition of 0·5 m-mole of fructose, 4·4 μmoles/g liver protein (Fig. 2) or approximately 5·3 μmoles/total liver (mean total liver protein was 1·2 g), agrees closely with the estimated loss of adenine nucleotides. These calculations suggest that degradation of preformed purines is the main mechanism of fructose-induced increase in uric acid and allantoin production.

We have previously postulated that the mechanism of fructose-induced depletion of liver adenine nucleotides is dependent on the rapid phosphorylation of fructose, which is associated with the accumulation of fructose-1-phosphate and depression of hepatic P_i levels.[2]

Fructose is phosphorylated mainly by fructokinase (ATP:D-fructose 1-phosphotransferase, EC 2.7.1.3),[17] and calculations of the rate of phosphorylation in rat liver slices,[18] homogenates,[19] and in the isolated perfused liver[20] are in good agreement with the observed *in vitro* activity of the enzyme (2·2 to 3·12 μmoles/min per g fresh weight).[21,22] Estimation of the disappearance rate of fructose after the addition of 0·5 m-mole in our perfusion experiments yields a value of 3·1 μmoles/min per g fresh weight.

The cleavage of fructose-1-phosphate by liver aldolase (ketose-1-phosphate aldehyde lyase, EC 4.1.2.7) is considered to be rate-limiting in hepatic fructose metabolism.[23] The accumulation of fructose-1-phosphate in rat liver tissue 10 min after the administration of approximately 0·3 m-mole/100 g body weight of fructose has been found to be of the order of 1·7–3·8 μmoles/g fresh weight.[24] This agrees well with our data on liver P_i depletion, 2·07 μmoles/g fresh weight 10 min after an injection of the somewhat higher dose of 1·0 m-mole (Table 1).

The rapid phosphorylation of L-sorbose by rat liver fructokinase[22] explains the effect of this monosaccharide on hepatic ATP and P_i levels (Table 3). The moderate effect obtained with sorbitol is evidently due to the fact that sorbitol is metabolized

as fructose after the initial dehydrogenation catalyzed by L-iditol dehydrogenase (L-iditol:NAD oxidoreductase, EC 1.1.1.14). On the other hand, the other monosaccharides tested are phosphorylated much more slowly than fructose or sorbose, for instance the rate for glucose has been found to be only one-fifth to one-third of that for fructose.[18,25] Consequently, neither rapid utilization of ATP nor sequestration of P_i is likely to occur to a similar extent. A moderate P_i depression did, however, occur after glucose and mannose administration (Table 3).

The moderate ATP depletion in kidney tissue and unchanged levels in heart tissue after fructose administration (Table 4) are not unexpected findings, if the organ distribution of fructokinase is considered. The enzyme is present in the kidney but absent in the heart,[22] and the phosphorylation of fructose by hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) is very slow, if glucose is present.[17]

The important role of fructose phosphorylation in the causation of adenine nucleotide depletion is indirectly supported by studies on fructose-induced hyperuricemia in man.[1] In a patient with essential fructosuria, unable to phosphorylate fructose, no change in serum or urine uric acid levels was observed after fructose administration. On the other hand, in patients with hereditary fructose intolerance, in which only the initial phosphorylation step proceeds normally, the response of uric acid to fructose did not differ from that of healthy subjects.

Our data do not allow conclusions about the initial pathway of adenine nucleotide degradation after fructose administration. The main question is, as in the case of an analogous phenomenon caused by anoxia,[26,27] whether AMP is deaminated prior to dephosphorylation or *vice versa*. The known sensitivity of AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) to inhibition by P_i at relatively low concentrations[28–30] suggests that an acceleration of this enzymatic reaction occurs, as the intracellular P_i levels fall after fructose administration. However, multiple regulatory factors are involved in AMP catabolism,[31] and speculation on the basis of the present studies is unwarranted.

Anoxia deserves consideration in the interpretation of our results also as a well-known source of error in adenine nucleotide determinations.[32] Such an error might arise either in the course of sampling or during the preceding experimental procedures. However, our findings cannot be explained on methodological grounds, because our control values (Table 1) agree well with those reported by others,[32] and as the alterations produced by fructose were not reproduced by other test compounds despite identical experimental procedures (Table 3). Also in liver perfusion studies, oxygenation is the main factor determining the liver ATP levels, but these have been shown to be relatively well maintained.[33] Although we have not measured liver ATP levels in our perfusion experiments, the excellent capacity of our preparation to phosphorylate fructose (*vide supra*) and to synthesize urea,[7] both processes being ATP dependent, is evidence against unphysiological conditions with regard to ATP synthesis.

Whatever the detailed processes associated with the effect of fructose on liver adenine nucleotides may be, the implications of the phenomenon are obvious. Significant transient depletion was demonstrated in rats *in vivo* with 0·25 m-mole of fructose. In terms of body weight, this corresponds to 0·25 g/kg, which must be regarded as a pharmacologic dose in man. Intravenous administration of fructose has several applications in clinical medicine, and it is a common and well-absorbed dietary constituent. Deleterious effects are probably unimportant in the majority of

cases, as the depletion of liver adenine nucleotides is so transient. However, certain clinical disorders, for instance anoxia, may contribute to the metabolic alterations described and demand reconsideration of therapeutic principles.

Acknowledgements—The Foundation for Pediatric Research in Finland provided the laboratory facilities for these studies. Financial support was granted by the Signe and Ane Gyllenberg Foundation Helsinki, Finland, and by Huhtamäki-Yhtymä, Lääketehdas Turku, Finland.

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