The Mechanism of Adenosine Triphosphate Depletion in the Liver after a Load of Fructose

A KINETIC STUDY OF LIVER ADENYLATE DEAMINASE

By GEORGES VAN DEN BERGHE, MIGUEL BRONFMAN,* ROLANDE VANNESTE
and HENRI-GÉRY HERS

*Laboratoire de Chimie Physiologique, Université de Louvain and International Institute of Cellular
and Molecular Pathology, 1200 Brussels, Belgium*

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1. The hepatic concentration of several nucleotides and metabolites was measured during the first few minutes after an intravenous load of fructose to mice. The first changes, observed at 30s, were a decrease in the concentration of P_i and a simultaneous accumulation of fructose 1-phosphate. The decrease in the concentrations of ATP and GTP proceeded more slowly. An increase in the concentration of IMP was detected only after 1 min and could therefore not be considered to be the cause of the accumulation of fructose 1-phosphate. 2. To explain the temporary burst of adenine nucleotide breakdown that occurs after a load of fructose, the kinetics of AMP deaminase (EC 3.5.4.6) from rat liver were reinvestigated at physiological (0.2 mM) concentration of substrate. For this purpose, a new radiochemical-assay procedure was developed. At 0.2 mM-AMP a low activity could be measured, which was more than 90% inhibited by 5 mM- P_i . ATP (3 mM) increased the enzyme activity over 200-fold. P_i alone did not influence the ATP-activated enzyme, but 0.5 mM-GTP caused a 60% inhibition. The combined effect of both inhibitors at their physiological concentrations reached 95%. 3. It is proposed that the rapid degradation of adenine nucleotides that occurs after a load of fructose is caused by a decrease in the concentration of both inhibitors, P_i and GTP, soon counteracted by the decrease in the concentration of ATP. 4. Some of the kinetic parameters of liver AMP deaminase were computed in terms of the concerted transition theory of Monod, Wyman & Changeux (1965) (*J. Mol. Biol.* 12, 88–118).

The continuous generation of high-energy phosphate groups, mainly in the form of ATP, requires a closely regulated adenine nucleotide pool in the cell. This pool consists of ATP, ADP and AMP, maintained in equilibrium by adenylate kinase (EC 2.7.4.3). It is continually subjected to catabolism, since two mechanisms, described in Scheme 1, can potentially convert AMP into inosine and ultimately into uric acid or allantoin in the liver; indeed, the amino group of AMP could be removed either before (by AMP deaminase; AMP aminohydrolase, EC 3.5.4.6) or after (by adenosine deaminase, EC 3.5.4.4) the hydrolysis of the phosphate group. Calculations from the amount of uric acid excreted by man (less than 600 mg/day) and data obtained by catheterization of the hepatic vein (Grunst *et al.*, 1975) show that as little as 3–5 nmol of uric acid is formed/min per g of liver under basal conditions; in contrast, an intravenous load of fructose can increase this rate by one or two orders of magnitude, depending on whether the hepatic uric acid production (Grunst *et al.*, 1975) or the loss of adenine

nucleotides (Mäenpää *et al.*, 1968) is measured. The slower formation of uric acid as compared with the disappearance of adenine nucleotides is in agreement with the known accumulation of IMP under these experimental conditions (Woods *et al.*, 1970). This burst of degradation of adenine nucleotides after a load of fructose is of short duration, and stops when the concentration of ATP has decreased to around 1 mM (Mäenpää *et al.*, 1968).

The purpose of the work in the present and in the following paper (Van den Berghe *et al.*, 1977) was to clarify which of the two possible pathways of AMP degradation depicted in Scheme 1 actually operates in the liver and how it is regulated. We show that 5'-nucleotidase (EC 3.1.3.5) is virtually inactive on AMP at physiological concentration (Van den Berghe *et al.*, 1977); accordingly, the determination of metabolites in the liver after the administration of fructose to mice is in agreement with the hypothesis that all the catabolism of AMP operates via AMP deaminase. The kinetic properties of this enzyme have been reinvestigated by means of a radiochemical method which permits measurement of

* Present address: Instituto de Ciencias Biológicas, Universidad Católica de Chile, Santiago, Chile.

Scheme 1. Pathway of AMP degradation (after Woods *et al.*, 1970)

enzymic activity at low (0.02 mM) concentrations of AMP. It is concluded that the two major inhibitors of the enzyme are P_i and GTP, and that the decreased concentration of these two inhibitors after a load of fructose explains the temporary burst of uric acid formation; the subsequent decrease in the concentration of ATP, which is a major stimulator of AMP deaminase, presumably arrests the degradation process. Part of this work has been published in a preliminary form (Van den Berghe *et al.*, 1975).

Materials and Methods

Chemicals

Non-radioactive nucleotides, phosphoenolpyruvate, succinate, CoA and NADH were obtained from Sigma Chemical Co., St Louis, MO, U.S.A.; [$8\text{-}^{14}\text{C}$]IMP (specific radioactivity 28 mCi/mmol) was from Schwarz/Mann, Orangeburg, NY, U.S.A.; [$8\text{-}^{14}\text{C}$]AMP (specific radioactivity 60 mCi/mmol) and [$8\text{-}^3\text{H}$]GTP (specific radioactivity 17 Ci/mmol) were from The Radiochemical Centre, Amersham, Bucks., U.K. DE-52 DEAE-cellulose was purchased

from Whatman Biochemicals, Maidstone, Kent, U.K., and Dowex AGI (X8; 200–400 mesh; Cl^- form) was from Bio-Rad Laboratories, Richmond, CA, U.S.A. The resin was converted into the formate form with 5 M-ammonium formate. All other chemicals were analytical grade.

Enzymes

AMP deaminase was purified from rat liver by the method of Smith & Kizer (1969), with the following modifications: solid Li_2SO_4 was added to a final concentration of 1 M; heat denaturation was performed at 45°C for 15 min; column chromatography was accomplished on DE-52 DEAE-cellulose without addition of dextran. Fractions containing enzyme activity were pooled and used as such. The gel-filtration step was omitted. The enzyme preparation was free of myokinase and 5'-nucleotidase activity, measured as described by Adam (1963) and Avruch & Wallach (1971) respectively. The enzyme was stored at -20°C for up to 2 months. After this time-interval, loss of activity occurred.

Hexokinase (EC 2.7.1.1), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), adenylate kinase (myokinase), pyruvate kinase (EC 2.7.1.40), lactate dehydrogenase (EC 1.1.1.27), adenosine deaminase, succinyl-CoA synthetase (EC 6.2.1.4), xanthine oxidase (EC 1.2.3.2) and purine nucleoside phosphorylase (EC 2.4.2.1) were purchased from Boehringer G.m.b.H., Mannheim, Germany. 5'-Nucleotidase from *Crotalus adamanteus* venom was obtained from BDH Chemicals, Poole, Dorset, U.K. Rabbit muscle phosphorylase *b* was purified as described by Fischer & Krebs (1958).

Animal experiments

These were performed with normally fed Naval Medical Research Institute mice weighing about 20 g. Fructose was injected as a 20% (w/v) solution via a tail vein, at a dose of 2.5 mg/g body wt. The animals were anaesthetized by the intravenous injection of sodium pentobarbital (75 mg/kg body wt.) approx. 1 min before excision of the liver. The liver was quick-frozen as described by Wollenberger *et al.* (1960) and stored at -20°C until processed.

Sampling of liver

Approx. 400 mg of frozen liver was broken up and immediately homogenized in 3 ml of ice-cold 10% (w/v) HClO_4 in a Potter-Elvehjem tube, fitted with a Teflon pestle. After the addition of 1 ml of water, the slurry was centrifuged at 500 g for 20 min at 4°C ; the resulting supernatant was neutralized with 0.4 M-imidazole/2 M-KOH/0.4 M-KCl (Lowry & Passonneau,

1972), and clarified again by centrifugation (500g for 20min). This supernatant was used for the determination of ATP, ADP, AMP, P_i , fructose 1-phosphate and adenosine. Another frozen liver sample was similarly homogenized in 3ml of 10% (w/v) ice-cold trichloroacetic acid, containing a tracer amount of $[8-^{14}C]IMP$ and $[8-^3H]GTP$. The supernatant obtained after the addition of 1ml of water and centrifugation (500g for 20min) was extracted with 5×5 vol. of ether to remove trichloroacetic acid. The last traces of ether were eliminated by heating the sample for 2min at $100^\circ C$. Part of this solution was used as such for the determination of the sum of $GDP+GTP$. Another portion was concentrated under vacuum, spotted on Whatman no. 1 paper and developed for 60h by descending chromatography with isobutyric acid/1M- NH_3 /0.1M-EDTA (125:75:2, by vol., Krebs & Hems, 1953). This procedure was used for the isolation of GTP and IMP; average distances run from the starting line were 10 and 25cm respectively. The areas corresponding to the nucleotide standards were cut out and eluted with 2ml of water. These eluates were used for both the spectrophotometric assay and the determination of radioactivity for the calculation of the recovery of the nucleotide.

Analytical methods

ATP was determined by the method of Lamprecht & Trautschold (1963). ADP and AMP were measured as described by Adam (1963). P_i was determined as described by Weil-Malherbe & Green (1951). The concentrations of free fructose and of fructose 1-phosphate were measured as described previously (Van den Berghe *et al.*, 1973). Adenosine was measured by the decrease in A_{265} after addition of adenosine deaminase (Kalckar, 1947b). Xanthine and hypoxanthine, inosine and IMP were determined by the increment in $A_{292.5}$ through the successive addition of xanthine oxidase, purine nucleoside phosphorylase and 5'-nucleotidase, by the method of Kalckar (1947a). In unpurified $HClO_4$ or trichloroacetic acid liver extracts, the amounts of xanthine, hypoxanthine and inosine were negligible. After addition of 5'-nucleotidase, the absorbance tended to increase continuously, presumably because of the presence of adenosine deaminase in the latter enzyme preparation. For this reason, the assay was used for the determination of IMP after its chromatographic isolation. The sum of GTP plus GDP was measured in trichloroacetic acid extracts by following the rate of formation of pyruvate from phosphoenolpyruvate in a cycling system, by using guanine nucleotide-specific succinyl-CoA synthetase and pyruvate kinase (Cha & Cha, 1970). GTP was determined by the same procedure after paper-chromatographic isolation.

Density-gradient centrifugations

These were carried out at $2^\circ C$ for 16h in linear 5–20% (w/v) sucrose gradients (5ml) as described by Martin & Ames (1961), by using a SW 39 rotor in a Spinco model L 2 ultracentrifuge. The gradients contained 50mM-glycylglycine buffer, pH7.4, 100mM-KCl, 5mM-MgCl₂, and the additions described in the Results section. Molecular weights were estimated from the ratios of mobilities, with lactate dehydrogenase (mol.wt. 140000) and muscle phosphorylase b (mol.wt. 185000) as standards.

Assay of AMP deaminase

The activity of the purified enzyme was measured at $20^\circ C$ by the rate of formation of radioactive IMP from $[^{14}C]AMP$. The incubation medium contained 50mM-glycylglycine buffer, pH7.4, 100mM-KCl, 5mM-MgCl₂, various concentrations of $[^{14}C]AMP$ and other additions, as indicated, in a total volume of 0.2ml. The reaction was terminated by the successive additions of 0.1ml of 0.125M-formic acid and 1ml of 0.1mM-carrier IMP. This mixture was applied to a column (0.5cm $\times$ 5cm) of Dowex AG 1 (X8; 200–400 mesh; formate form) connected to a glass reservoir. The column was first washed with 100ml of 0.125M-formic acid to remove the residual AMP. IMP was eluted with 6ml of 8M-formic acid/1M-ammonium formate. A 1ml portion of this eluate was counted for radioactivity with 10ml of the scintillation mixture of Patterson & Greene (1965). The amount of IMP formed was calculated by taking into account the slight (16%) quenching exerted by the high ionic concentration.

In preliminary experiments, it was verified that the washing procedure removed up to $1\mu mol$ of $[^{14}C]AMP$ from the column, resulting in a blank value for the eluate of less than 2% of the radioactivity of the substrate. The radioactive product, appearing in the 8M-formic acid/1M-ammonium formate eluate after incubation with AMP deaminase, was shown to migrate in the same position as authentic IMP in the paper-chromatography system described above. When radioactive IMP was added to the incubation medium, a small contaminant was recovered in the 0.125M-formic acid washings, even on successive columns. Recovery of $[^{14}C]IMP$ in the eluate was more than 90%. The formation of radioactive IMP from $[^{14}C]AMP$ by the enzyme preparation was linear with time and the amount of enzyme.

Results

Influence of the administration of fructose on metabolite concentrations in mouse liver

The rapid accumulation of fructose 1-phosphate and depletion of P_i and ATP observed in the liver of mice after a load of fructose (Fig. 1) are in accord

Fig. 1. Effect of a fructose load on metabolites in mouse liver. Values shown are means $\pm$ S.E.M. of four animals.

Table 1. Influence of a fructose load on the concentration of GTP in mouse liver

Values shown are means $\pm$ S.E.M. There were four or five animals per experimental point. Statistical significance versus zero-time value: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$.

Time after fructose (min)	GTP ($\mu\text{mol/g}$ of liver)	
	Exp. 1	Exp. 2
0	0.59 ± 0.06	0.57 ± 0.06
0.5	0.52 ± 0.07	$0.47 \pm 0.02^{**}$
1	0.47 ± 0.04	$0.47 \pm 0.02^{**}$
1.5	$0.38 \pm 0.06^*$	—
2	$0.38 \pm 0.02^{**}$	$0.43 \pm 0.03^{***}$

with previous findings in the rat (Mäenpää *et al.*, 1968; Raivio *et al.*, 1969; Yu *et al.*, 1974). The accumulation of IMP after the administration of fructose, described by Woods *et al.* (1970), clearly followed the build-up of fructose 1-phosphate. The concentration of adenosine, which was lower than $0.1 \mu\text{mol/g}$ in the liver of control mice, was not increased by a fructose load. Table 1 shows that the ketose also induced a significant decrease in the hepatic concentration of GTP. The sum of [GTP] plus [GDP] was not modified by this treatment (results not shown).

Fig. 2. Influence of the concentration of AMP on the rate of its deamination in the absence ($\circ$) and in the presence ($\bullet$) of 3 mM-ATP

Kinetic study of AMP deaminase

Kinetics at variable substrate concentration. Although the new radiochemical assay procedure was devised for the measurement of AMP deaminase at low substrate concentration, we have also used it to check that the basal kinetic properties of our enzyme preparation were similar to those previously described by others (for a review, see Zielke & Suelter, 1971). Fig. 2 shows that in the absence of effectors, the saturation curve for AMP was sigmoidal, with $s_{0.5} = 9 \text{ mM}$. Calculation of the Hill coefficient gave a value of 1.6. In the presence of 3 mM-ATP, the affinity for AMP was greatly increased ($K_m = 0.25 \text{ mM}$) and the kinetics were changed from sigmoidal to hyperbolic. Another effect of a physiological concentration of ATP was to abolish the well-known inhibitory effect of P_i . It had indeed been shown by Nikiforuk & Colowick (1956) that this inhibitory effect was particularly important at low substrate concentration, and accordingly we have found 90% inhibition by 5 mM- P_i at 0.2 mM-AMP (results not shown). Fig. 3 shows that at substrate concentrations up to 1 mM, including the physiological range, there was no effect of P_i in the presence of 3 mM-ATP, but 0.5 mM-GTP was inhibitory. On addition of all three modulators, the enzyme was profoundly inhibited, its activity being only slightly higher than in the absence of effectors.

Kinetics at 0.2 mM-AMP. The physiological concentration of 0.2 mM-AMP has been chosen for a series of experiments designed to analyse the combined effects of the known natural effectors ATP, P_i and GTP. Fig. 4 shows that the saturation curve of ATP was sigmoidal and that the enzyme was

Fig. 3. Influence of various effectors on the activity of AMP deaminase

The activity of the enzyme was measured in the absence of effectors (○) and with the addition of: 3mM-ATP (●); 3mM-ATP and 5mM- P_i (▲); 3mM-ATP and 0.5mM-GTP (■); 3mM-ATP, 5mM- P_i and 0.5mM-GTP (▼).

Fig. 5. Influence of the concentrations of GTP and P_i on the activity of AMP deaminase measured in the presence of 3mM-ATP
AMP concentration was 0.2mM.

Fig. 4. Influence of the concentration of ATP on the activity of AMP deaminase in the absence (○) and in the presence (●) of 5mM- P_i

The activity of the enzyme was measured at 0.2mM-AMP.

Fig. 6. Influence of the concentrations of GTP and ATP on the activity of AMP deaminase measured in the presence of 2.5mM- P_i
AMP concentration was 0.2mM.

practically saturated with the ligand at concentrations higher than 1mM. The inhibition by P_i was only apparent at low concentrations of ATP (below 1.5–2mM, according to the batch of enzyme

used). The effect of increasing concentrations of GTP in the presence of various concentrations of ATP and P_i is shown in Figs. 5 and 6; the inhibitory action of GTP at physiological concentrations (0.3–0.6mM)

Fig. 7. Linearization of the simplified saturation function. The data of Fig. 2, obtained in the absence of ATP, were plotted according to eqn. (2).

is reinforced by increased concentrations of P_i (Fig. 5) and by decreased concentrations of ATP (Fig. 6). At physiological concentrations of all three effectors (3 mM-ATP, 0.5 mM-GTP, 5 mM- P_i) the combined inhibitory effects reached 95%. ADP at up to 1 mM concentration did not influence the enzyme activity in the presence of 3 mM-ATP and 5 mM- P_i , in the absence as well as in the presence of 0.5 mM-GTP. IMP (1 mM) provoked a 25% inhibition of the enzyme in the presence of 3 mM-ATP and 5 mM- P_i (not shown).

Computation of the data. Some of the data presented have been computed in terms of the concerted transition theory of Monod *et al.* (1965), assuming a perfect K system. According to this model, an allosteric protein, made of n oligomers, can exist in two conformational states, R and T. State R displays high affinity for the substrate and the activator(s), whereas state T has affinity for the inhibitor(s). K_R and K_T are the microscopic dissociation constants of a ligand bound to a stereospecific site in the R and the T states respectively. The hyperbolic substrate-saturation curve obtained in the presence of 3 mM-ATP (Fig. 3) indicates that, at this concentration of the activator, the equilibrium of the allosteric transition is completely shifted towards the R conformation. K_R (AMP) is thus equal to the apparent K_m measured under these conditions, namely 0.25 mM.

Since the affinity of the enzyme for AMP is very much increased in the presence of ATP, one can assume an exclusive binding of the substrate to the R state ($c = K_R/K_T = 0$). Under these conditions and in the absence of allosteric effectors, the saturation function simplifies to:

$$\bar{Y}_s = \frac{v}{V} = \frac{\alpha(1+\alpha)^{n-1}}{L + (1+\alpha)^n} \quad (1)$$

Fig. 8. Variation of the quotient function $\bar{Q}$ with respect to the concentration of ATP.

The data of Fig. 4 were plotted according to eqn. (3), taking $n = 2$ in the absence of P_i (○) and $n = 8$ in the presence of 5 mM- P_i (●).

where $\bar{Y}_s$ is the fractional saturation of the enzyme, V the maximum velocity, n the number of oligomers, L the allosteric constant and $\alpha = [\text{AMP}]/K_R$ (AMP). We have linearized this equation as follows:

$$\log\left(\frac{V'-v}{v}\right) = \log L - n \log(1+\alpha) \quad (2)$$

where

$$V' = \frac{V \cdot \alpha}{1 + \alpha}$$

V' is the maximum velocity that can be reached in the presence of a given concentration of substrate, when all the enzyme molecules are in the R conformation (Blangy *et al.*, 1968). Fig. 7 shows a plot of this equation using the data of Fig. 2. It can be calculated that n is approx. 2, and L , the allosteric constant, 1000. The same values were obtained by the linearization procedure described by Horn & Börnig (1969).

As described by Blangy *et al.* (1968) a plot of $(v/V'-v)^{1/n}$ against the concentration of the activator, at fixed concentration of the substrate, according to the quotient function:

$$\bar{Q}^{1/n} = \left(\frac{v}{V'-v}\right)^{1/n} = \frac{(1+\alpha)}{L^{1/n}} \left(1 + \frac{[\text{ATP}]}{K_R(\text{ATP})}\right) \quad (3)$$

should give a straight line, which intercepts the abscissa at $-K_R(\text{ATP})$, provided that the value of n has been correctly chosen. The experimental values of Fig. 4 have been plotted according to this

equation in Fig. 8, which shows that, taking $n = 2$, the values obtained in the absence of P_i fit well to a straight line. The intercept with the abscissa provides $K_R(ATP) = 7 \mu M$. In the presence of 5 mM- P_i , however, taking $n = 2$ gave an upward curvature (not shown), but a straight line could be drawn by introducing $n = 8$. Deviation from linearity could be explained by transformation of the enzyme from a dimeric to an octameric form, induced by P_i .

Sedimentation in sucrose density gradients. To establish whether P_i influenced the aggregation state of AMP deaminase, 0.2 mg of the enzyme preparation was sedimented in sucrose density gradients with the addition of either 3 mM-ATP or 5 mM- P_i . In both conditions, the enzyme sedimented at a rate corresponding to a mol.wt. of approx. $280\,000 \pm 5\,000$ (mean $\pm$ S.E.M. of seven measurements).

Discussion

Regulation of AMP deaminase

Several investigations have been previously devoted to the kinetic properties of AMP deaminase from various mammalian tissues, namely the brain (Cunningham & Lowenstein, 1965; Setlow & Lowenstein, 1967, 1968; Lee & Wang, 1968), the muscle (Smiley & Suelter, 1967; Ronca-Testoni *et al.*, 1970) and the liver (Smith & Kizer, 1969; Chapman & Atkinson, 1973). With regard to the liver enzyme, earlier work has revealed the sigmoidal property of the substrate-affinity curve (Smith & Kizer, 1969; Chapman & Atkinson, 1973), the stimulatory effect of ATP (Setlow *et al.*, 1966) and the inhibition by P_i (Chapman & Atkinson, 1973) and by GTP (Setlow *et al.*, 1966).

We have confirmed these various properties and analysed them at physiological concentration of the substrate (0.2 mM) and effectors (3 mM for ATP, 0.5 mM for GTP, 5 mM for P_i). At 0.2 mM-AMP the enzyme displays very little activity, but 3 mM-ATP increases this value over 200-fold. We have shown that the inhibition by phosphate is very much counteracted by ATP and is almost non-existent at the physiological concentration of the nucleoside triphosphate. This indicates that a decrease in P_i concentration in itself is not a sufficient explanation for the rapid degradation of adenine nucleotides that occurs after a load of fructose. GTP appears to be a more powerful inhibitor of the ATP-activated enzyme, but it is only the combined effect of P_i and GTP that allows a nearly complete inhibition of the deaminase under physiological conditions.

The mathematical study of our kinetic data had suggested that the enzyme may exist as a dimer in the presence of ATP, whereas P_i would provoke the aggregation to an octameric form. This interpretation was, however, not confirmed by the results of the

gradient-centrifugation experiments. The alternative interpretation for the observed departure from linearity of the quotient function (eqn. 3) with $n = 2$, would be, according to Blangy *et al.* (1968), a non-exclusive binding of the activator. A non-exclusive binding of ATP in the presence of P_i is, however, difficult to reconcile with the fact that the substrate-saturation curve of the ATP-activated enzyme follows normal hyperbolic kinetics in the presence as well as in the absence of P_i . We therefore tentatively conclude that P_i acts by interfering with the binding of low concentrations of ATP to the R form and by augmenting the affinity of GTP for the T form.

The mol.wt. of 280 000 which we have calculated for the liver enzyme is in close agreement with the value of 270 000 reported for the muscle enzyme (Wolfenden *et al.*, 1968). The latter enzyme has been shown to be composed of four subunits (Scopes & Penny, 1971; Boosman *et al.*, 1971), a finding in accordance with the demonstration of a minimum of four binding sites for ATP (Tomozawa & Wolfenden, 1970) and of a Hill coefficient of 4.3 for the brain enzyme in the absence of alkali-metal ions (Setlow & Lowenstein, 1967). The latter authors have, however, found a Hill coefficient of 1.6 in the presence of 150 mM-LiCl, which is identical with the value obtained by us in the presence of 100 mM-KCl. The finding of two binding sites for GTP by Tomozawa & Wolfenden (1970) is a further indication that the molecular structure of AMP deaminase probably does not fit a simple model.

Sequence of events after a load of fructose

The determination of metabolite concentrations in the liver during the early periods after a load of fructose indicated that, as expected, the accumulation of fructose 1-phosphate and a simultaneous decrease in P_i and ATP were the first changes to occur. Indeed, a high concentration of fructose is assumed to affect only the activity of fructokinase (EC 2.7.1.3) and the phosphate of fructose 1-phosphate must necessarily come from ATP, which itself is rapidly regenerated at the expense of P_i .

The rise in IMP concentration, initially described by Woods *et al.* (1970), provides strong evidence that the pathway of AMP degradation (Scheme 1) occurs through AMP deaminase in the liver. No adenosine could be detected under the same conditions. The possibility that it might have been formed at a significant rate by 5'-nucleotidase, but not have accumulated because of rapid metabolism, appears unlikely in view of the kinetic properties of the soluble 5'-nucleotidase described in the following paper (Van den Berghe *et al.*, 1977).

The diminution in the concentration of GTP, superimposed on the decrease in P_i , is an important

factor that may release the inhibition of AMP deaminase. The decrease of the concentration of the guanine nucleotide after a fructose load might, in principle, be due to several mechanisms. Since the decrease in the concentration of ATP occurs at a higher rate than the decline of GTP, it appears that the guanine nucleotide is not in a rapid equilibrium with ATP, notwithstanding the high activity of nucleoside diphosphate kinase (EC 2.7.4.6) in the liver (Cheng *et al.*, 1973). Studies by Adelman *et al.* (1967) have ruled out GTP as the phosphoryl donor in the reaction catalysed by ketohexokinase. A likely explanation for the diminution of the concentration of GTP is the finding that triokinase (EC 2.7.1.28) can use this nucleoside triphosphate at a rate which is about one-tenth of that of the utilization of ATP (Frandsen & Grunnet, 1971). Considering the relatively low concentration of GTP in the liver cell and the finding that the sum of [GTP]+[GDP] did not change, it seems that this mechanism may be a sufficient explanation for the observed change in concentration of the guanine nucleotide.

Already from the work of Mäenpää *et al.* (1968) it appears that the decrease in the concentration of the adenine nucleotides was terminated after about 2 min, when the concentration of ATP reached about 1 mM, whereas the increase in uric acid and allantoin in the blood continued for a much longer time. This can be interpreted either by assuming that at that time a new steady state between nucleotide synthesis and degradation has been reached, or that degradation has been stopped and that uric acid is formed from IMP which has accumulated in the liver. Although stimulation of purine synthesis *de novo* by fructose infusion has been reported (Raivio *et al.*, 1975), it appears from our kinetic data that the fall in [ATP] to a value close to 1 mM enhanced the inhibitory action of P_i and GTP, and presumably greatly decreased the degradation of nucleotides at that time.

Accumulation of fructose 1-phosphate

Woods *et al.* (1970) have discussed the point that the accumulation of fructose 1-phosphate in the liver after a load of fructose cannot be attributed to a lower activity of liver aldolase (EC 4.1.2.13) as compared with that of fructokinase. Further, they have concluded that the accumulation of IMP, causing an inhibition of aldolase, was the explanation for the build-up of the fructose derivative. Our finding that the accumulation of IMP occurs only during the second minute after intravenous administration of the ketose, when the concentration of fructose 1-phosphate has already reached its maximum, contradicts this interpretation.

From the value of the equilibrium constant of aldolase for the splitting of fructose 1-phosphate into

glyceraldehyde and dihydroxyacetone phosphate ($2.8 \times 10^{-6} M$, according to Lehninger *et al.*, 1955) and from the concentration of these intermediates found in the liver after the administration of fructose by Heinz & Junghänel (1969), one can calculate that the mixture is at thermodynamic equilibrium. It is thus not possible to invoke an inhibition of aldolase to explain the initial accumulation of fructose 1-phosphate. We therefore propose that this phenomenon is due to the fact that both fructokinase and aldolase act more rapidly than the metabolic pathways that convert triose phosphates into pyruvate and glucose. It seems indeed that the maximum velocity for each of these two pathways does not exceed 3–4 μmol of triose phosphate converted/min per g of liver (Exton & Park, 1967, Woods *et al.*, 1970), so that the additive rate of both is still lower than the potential activity of fructokinase which can be as high as 12 $\mu\text{mol}/\text{min}$ per g of liver according to Fig. 1.

Catabolism of adenine nucleotides and the pathogenesis of gout

From the work presented in this and the following paper (Van den Berghe *et al.*, 1977), it appears that, in the liver, adenine nucleotides are degraded exclusively by the pathway involving the hydrolytic deamination of AMP, followed by the hydrolysis of the phosphate group by the soluble 5'-nucleotidase. The first of these two enzymic steps is regulated by the concentrations of ATP, GTP and P_i . The activity of this catabolic pathway can be enormously increased after a load of fructose, apparently because of a change in the concentration of these effectors and only slightly because of an increase in the concentration of substrate. It must therefore be pointed out that an increased formation of uric acid, as observed in hyperuricaemic subjects, could be secondary to disturbances of the complex regulation of AMP deaminase. These could be due to an anomalous enzyme or to a slight modification in the hepatic concentration of one of its effectors; one might for instance propose that gouty subjects might have a less active mechanism for the intracellular transportation of P_i or have a slightly decreased concentration of GTP. This would result in an increased rate of degradation of AMP, followed by an increased synthesis of adenine nucleotides *de novo*, since this metabolic pathway is tightly controlled by the intracellular concentration of AMP (see Wyngaarden & Kelley, 1972).

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