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Purine Catabolism in Isolated Rat Hepatocytes

INFLUENCE OF COFORMYCIN

Georges VAN DEN BERGHE, Françoise BONTEMPS and Henri-Géry HERS
Laboratoire de Chimie Physiologique, Université de Louvain and International Institute of Cellular and Molecular Pathology, UCL 75.39, avenue Hippocrate 75, B-1200 Bruxelles, Belgium

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1. The catabolism of purine nucleotides was investigated by both chemical and radiochemical methods in isolated rat hepatocytes, previously incubated with [^{14}C]adenine. The production of allantoin reached 32 ± 5 nmol/min per g of cells (mean ± S.E.M.) and as much as 30% of the radioactivity incorporated in the adenine nucleotides was lost after 1 h. This rate of degradation is severalfold in excess over values previously reported to occur in the liver *in vivo*. An explanation for this enhancement of catabolism may be the decrease in the concentration of GTP. 2. In a high-speed supernatant of rat liver, adenosine deaminase was maximally inhibited by 0.1 μM-coformycin. The activity of AMP deaminase, measured in the presence of its stimulator ATP in the same preparation, as well as the activity of the partially purified enzyme, measured after addition of its physiological inhibitors GTP and P_i, required 50 μM-coformycin for maximal inhibition. 3. The production of allantoin by isolated hepatocytes was not influenced by the addition of 0.1 μM-coformycin, but was decreased by concentrations of coformycin that were inhibitory for AMP deaminase. With 50 μM-coformycin the production of allantoin was decreased by 85% and the formation of radioactive allantoin from [^{14}C]adenine nucleotides was completely suppressed. 4. In the presence of 0.1 μM-coformycin or in its absence, the addition of fructose (1 mg/ml) to the incubation medium caused a rapid degradation of ATP, without equivalent increase in ADP and AMP, followed by transient increases in IMP and in the rate of production of allantoin; adenosine was not detectable. In the presence of 50 μM-coformycin, the fructose-induced breakdown of ATP was not modified, but the depletion of the adenine nucleotide pool proceeded much more slowly and the rate of production of allantoin increased only slightly. No rise in IMP concentration could be detected, but AMP increased manyfold and reached values at which a participation of soluble 5′-nucleotidase in the catabolism of adenine nucleotides is most likely. 5. These results are in agreement with the hypothesis that the formation of allantoin is controlled by AMP deaminase. They constitute further evidence that 5′-nucleotidase is inactive on AMP, unless the concentration of this nucleotide rises to unphysiological values.

The hepatic catabolism of the adenine nucleotides proceeds from AMP and terminates in the formation of uric acid or allantoin according to the species. Its initial pathway is generally thought to occur via two possible enzyme sequences: either a prior deamination by AMP deaminase, followed by dephosphorylation by 5′-nucleotidase, or a prior dephosphorylation by the latter enzyme, followed by deamination by adenosine deaminase. From studies performed with partially purified enzyme preparations (Van den Berghe *et al.*, 1977*a*,*b*), we have come to the conclusion that the initial step in the breakdown of physiological concentrations of AMP is catalysed by AMP deaminase. Indeed, the kinetic characteristics of the cytoplasmic 5′-nucleotidase preclude the dephosphorylation of physiological concentrations of AMP (around 0.1–0.2 mM), whereas the degradation of similar concentrations of IMP can occur freely. Other non-cytosolic 5′-nucleotidases do not qualify for a role in purine catabolism. It was also concluded that hepatic AMP deaminase is normally 95% inhibited by the concentrations of P_i and GTP prevailing in the liver cell, and that the well-known hyperuricaemic effect of

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fructose (Perheentupa & Raivio, 1967; Mäenpää *et al.*, 1968) is fully explained by deinhibition of the enzyme, caused by a decrease in the concentration of both inhibitors. The conclusion that the fructose-induced degradation of adenine nucleotides occurs only by way of AMP deaminase was also reached by Smith *et al.* (1977*b*), who found that isolated hepatocytes incubated with fructose did not form detectable amounts of radioactive adenosine from ^{14}C-labelled adenine nucleotides.

The purpose of the present work was to define further the limiting step of hepatic purine catabolism in relation to the postulated role of AMP deaminase in the pathogenesis of gout (Hers & Van den Berghe, 1979). The influence of an inhibition of this enzyme on the basal as well as the fructose-induced breakdown of adenine nucleotides was investigated in isolated hepatocytes. The nucleoside antibiotic coformycin {3β-D-ribofuranosyl-6,7,8-trihydroimidazo[4,5-*d*][1,3]diazepin-8(*R*)-ol} (Sawa *et al.*, 1967) was used for this purpose. This inosine analogue is a potent inhibitor of adenosine deaminase from various sources (Sawa *et al.*, 1967; Snyder & Henderson, 1973; Agarwal *et al.*, 1975). It also inhibits AMP deaminase from muscle (Agarwal & Parks, 1977; Henderson *et al.*, 1977), although higher concentrations are required than for adenosine deaminase. Part of this work has been published in a preliminary form (Van den Berghe *et al.*, 1979).

Materials and Methods

Chemicals

Coformycin was a generous gift of Professor H. Umezawa (Institute of Microbial Chemistry, Tokyo, Japan). Non-radioactive nucleotides and nucleosides were obtained from Boehringer G.m.b.H., Mannheim, Germany. Allantoin and uric acid were from Sigma Chemical Co, St Louis, MO, U.S.A. [8-^{14}C]Adenine (sp. radioactivity 62mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Cellulose thin-layer plates were from Carlo Erba, Milano, Italy, and polyethyleneimine–cellulose F thin-layer sheets were from E. Merck, Darmstadt, Germany. Sodium pentobarbital was from Serva, Heidelberg, Germany, and 4-dimethylaminobenzaldehyde was from U.C.B., Brussels, Belgium. K_2HPO_4 and KCl used for high-pressure liquid chromatography were obtained from Mallinckrodt, St Louis, MO, U.S.A.

Enzymes

Collagenase (grade II, EC 3.4.24.3), adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4), xanthine oxidase (EC 1.2.3.2), hexokinase (EC 2.7.1.1), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), adenylate kinase (myokinase, EC 2.7.4.3), pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27) were purchased from Boehringer.

Measurement of enzyme activities

The full expression of the inhibitory effect of coformycin on adenosine deaminase has been shown to require a prior incubation of the enzyme with the nucleoside antibiotic. This is related to the slow equilibration of the enzyme–inhibitor complex (Cha *et al.*, 1975). In all studies reported, coformycin was therefore preincubated for 15min with the enzyme preparation and the reaction started with the addition of substrate. Under these conditions, linear kinetics as a function of time were observed with all enzymes investigated.

Adenosine deaminase and AMP deaminase were measured in a high-speed supernatant of rat liver, obtained by centrifuging a 20% (w/v) homogenate prepared in 50mM-Tris/HCl buffer, pH7.4, containing 0.5% dithiothreitol, at 100000*g* for 60min. Both enzymes were assayed with an Aminco DW-2 dual-wavelength spectrophotometer, by the methods described by Schultz & Lowenstein (1976), with a light-path of 0.09mm. A 50mM-glycylglycine buffer, pH7.5, containing 100mM-KCl was used for the assay of AMP deaminase. Kinetic studies in the presence of P_i and GTP were performed with a radiochemical method on partially purified rat liver enzyme as described previously (Van den Berghe *et al.*, 1977*a*).

Liver homogenates prepared in 50mM-Tris/HCl buffer, pH7.4, were used for the determination of 5′-nucleotidase activity as described by Segal & Brenner (1960) and of nucleoside phosphorylase as described by Friedkin & Kalckar (1961). High-speed supernatants obtained by centrifuging homogenates prepared in 100mM-Tris/HCl buffer, pH8.0, at 100000*g* for 60min were used for the assay of guanase as described by Giusti (1974) and, of xanthine oxidase, after dialysis, as described by Rowe & Wyngaarden (1966). The activity of uricase was measured in homogenates prepared in 50mM-Tris/HCl buffer, pH8.0, by the method of Mahler (1963).

Experiments with isolated hepatocytes

Hepatocytes were isolated from adult (250–300g) fed male Wistar rats as described by Hue *et al.* (1975), except that the cells were suspended and incubated in Krebs–Henseleit (1932) bicarbonate buffer with the addition of 10mM-glucose. Samples (up to 12ml), containing about 70mg of cells/ml and various additions as indicated, were shaken (120strokes/min) in stoppered 50ml vials at 37°C. The gas phase was O_2/CO_2 (19:1). For the spectrophotometric determination of metabolites and the thin-layer-chromatographic separation of

nucleosides and nucleotides, portions of the hepatocyte suspension were pipetted into 0.5ml of ice-cold 10% (w/v) $HClO_4$. After centrifugation at 500*g* for 20min at 4°C, the resulting supernatant was neutralized with 3M-KOH/3M-$KHCO_3$, clarified again by centrifugation (500*g* for 20min) and stored at −20°C until the assays were performed. For the determination of GTP by high-pressure liquid chromatography, portions of the hepatocyte suspension were transferred into 0.5ml of ice-cold 10% (w/v) trichloroacetic acid. The supernatant obtained after centrifugation (500*g* for 20min) was extracted with 5 × 5vol. of ether to remove trichloroacetic acid. The last traces of ether were eliminated by heating the sample for 2min at 100°C.

Animal experiments in vivo

Fed male Wistar rats weighing about 300g were anaesthetized by the intraperitoneal injection of sodium pentobarbital (75mg/kg body wt.) and, when asleep, given O_2/CO_2 (19:1) to breathe. A liver sample was cut off and immediately quick-frozen as described by Wollenberger *et al.* (1960) while a ligature prevented excessive bleeding. Fructose was injected via a leg vein at a dose of 2.5mg/g body wt. and sequential samples were taken with the same technique at the time intervals indicated.

The frozen liver samples were broken up, immediately homogenized in 2ml of ice-cold 10% (w/v) trichloroacetic acid, and processed for the determination of GTP by high-pressure liquid chromatography as described for the hepatocytes.

Analytical methods

Allantoin was measured by the colorimetric method of Young & Conway (1942). In contrast with the data reported by those authors, glucose gave a colour reaction, which was subtracted by running an appropriate blank. Uric acid was determined as described by Liddle *et al.* (1959), ATP by the method of Lamprecht & Trautschold (1963), and ADP and AMP were measured as described by Adam (1963). Fructose 1-phosphate was measured by the method of Eggleston (1974). Purine nucleotides were separated on polyethyleneimine–cellulose thin-layer plates by stepwise development with sodium formate, after washing the plates twice in methanol/water (1:1, v/v) as described by Crabtree & Henderson (1971). One-dimensional chromatography of purine ribonucleosides and bases was performed on cellulose thin-layer plates that were developed twice in butan-1-ol/methanol/water/conc. NH_3 (sp.gr. 0.910) (60:20:20:1, by vol.). Purine compounds were located with u.v. light. Allantoin was detected by spraying the plates with a 5% (w/v) solution of 4-dimethylaminobenzaldehyde in 1M-HCl containing 50% ethanol (Cline & Fink, 1956). Although inosine was not separated from guanine and guanosine by this procedure, and allantoin migrated close to xanthosine and uric acid, the method was found satisfactory for the purposes of the experiments, since guanine and xanthine nucleotides are labelled only to a very limited extent by incubation of hepatocytes with [^{14}C]adenine, and the concentration of uric acid was negligible. The protein concentration was determined as described by Lowry *et al.* (1951), with bovine serum albumin as a standard, and a protein content of 20% was assumed when expressing the results per g of hepatocytes.

GTP was measured by high-pressure anion-exchange chromatography with a Hewlett–Packard 1084-B instrument equipped with a 25cm × 4.6mm Partisil 10-SAX column and monitored at 254nm. Separation was achieved by the use of a gradient with a low-concentration eluent of 0.007M-KH_2PO_4, pH4.0, and a high-concentration eluent of 0.25M-KH_2PO_4/0.5M-KCl, pH4.5, as described by Hartwick & Brown (1975). Peaks were identified and quantified by comparison with known standards of GTP.

The concentration of coformycin in isolated hepatocytes was determined as follows. Cells were incubated for various times with known concentrations of the nucleoside antibiotic. At the end of the incubation period, the suspension was poured into the separation tubes designed by Hems *et al.* (1975) and a $HClO_4$ extract was obtained and neutralized as described above. The concentration of coformycin in this extract was measured by its property to inhibit purified adenosine deaminase from calf intestine. When hepatocytes were incubated with 10μM-coformycin, the intracellular concentration of the inhibitor reached 50% of that in the medium after 2min and complete penetration occurred within 15min.

Results

Some characteristics of purine catabolism in isolated hepatocytes

Purine catabolism in isolated liver cells was measured as a whole by the production of allantoin. Uric acid could not be detected in the hepatocyte suspension, probably because of the high uricase activity in the liver cell. As shown in Figs. 1, 5 and 6, the concentration of allantoin present in the hepatocyte suspension at the beginning of the experiments was not negligible, even taking into account the fact that a 15min preincubation preceded zero time in all studies. This initial concentration of allantoin could be lowered, but only to a limited extent, by additional washings of the hepatocytes, which, however, resulted in a loss of cells and a decrease in their viability. It presumably results from

the catabolism of nucleotides during the isolation procedure.

As shown in Fig. 1, isolated hepatocytes produce allantoin at a linear rate for more than 60min. In basal conditions, the rate of production reached 32 ± 5 (mean $\pm$ S.E.M. of eight experiments) nmol/min per g of hepatocytes. The basal production of allantoin was not influenced by up to 64h of starvation of the donor rat. The addition to the hepatocyte suspension of millimolar concentrations of adenosine, guanine, guanosine (results not shown) and, as described in more detail below, of fructose increased the rate of production of allantoin several-fold above the basal rate.

A more specific estimation of the breakdown of adenine nucleotides was obtained by following the formation of radioactive allantoin in hepatocytes preincubated with labelled adenine as described by Smith *et al.* (1977*a*). Fig. 2 shows that when isolated hepatocytes were incubated with 1 μM-[^{14}C]adenine, all of the radioactivity was incorporated in the adenine nucleotides within 2min. Subsequently a linear decrease of the radioactivity in the adenine nucleotides was mirrored by an equivalent increase in the radioactivity in allantoin.

In agreement with the role of P_i in the control of liver AMP deaminase, a 30% decrease in the basal rate of allantoin production was observed when the concentration of P_i in the incubation medium was increased from 1 to 20mM (results not shown).

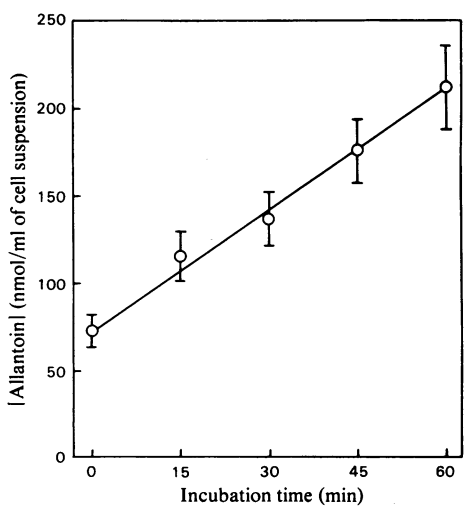

Fig. 1. *Formation of allantoin by isolated rat hepatocytes*

Allantoin was determined on cell suspensions containing 72 ± 5mg of cells/ml (mean $\pm$ S.E.M. of eight experiments) that had been preincubated for 15min before zero time.

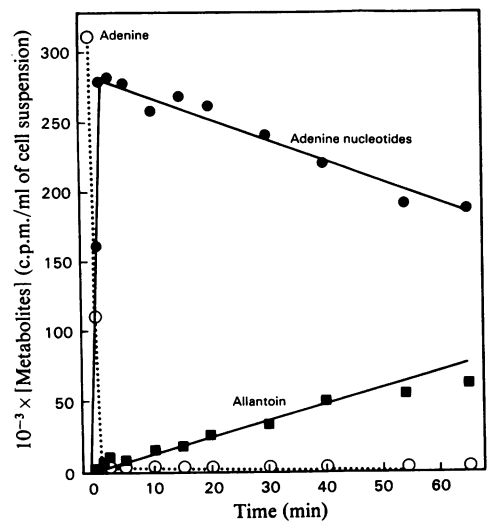

Fig. 2. *Metabolism of* [^{14}C]*adenine in isolated hepatocytes*

Cells were incubated in the presence of 1 μM-[^{14}C]adenine (0.2 μCi/ml of cell suspension) and processed as described in the Materials and Methods section.

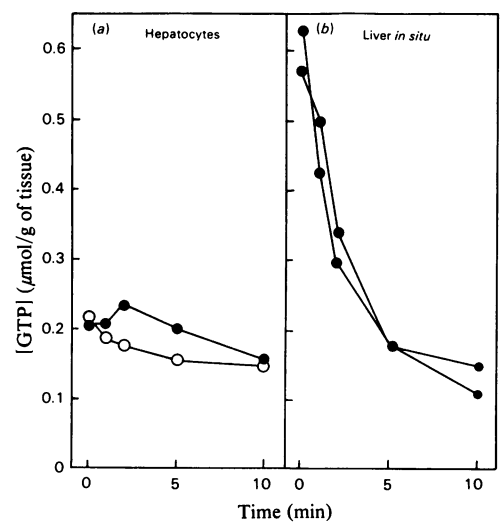

Fig. 3. *Influence of fructose on the concentration of GTP in isolated hepatocytes and in the liver in situ*

Isolated hepatocytes (*a*) were incubated in the absence (○) or in the presence of fructose (1 mg/ml) (●). Two anaesthetized rats (*b*) were injected intravenously with fructose (2.5 mg/g body wt.) and sequential biopsies were taken as described in the Materials and Methods section. The concentration of GTP was not significantly decreased in rats injected with physiological saline (0.9% NaCl).

In these conditions, the intracellular concentration of P_i has been reported to increase by 70% (Stermann *et al.*, 1978). An unexpected finding was that the concentration of GTP in the isolated hepatocytes was about 50% lower than in the intact liver (see Fig. 3). Attempts to increase the concentration of GTP in the hepatocytes after treatment of the donor rats or addition to the incubation medium of guanine or guanosine remained unsuccessful. Furthermore, in contrast with the observation that the administration of fructose to mice (Van den Berghe *et al.*, 1977*a*) and to rats *in vivo* (Fig. 3*b*) lowers the concentration of GTP in the liver, the low concentration of this nucleotide prevailing in isolated hepatocytes was not further decreased by the addition of fructose (Fig. 3*a*).

Influence of coformycin on hepatic enzyme activities

The concentration of coformycin required for maximal inhibition of AMP deaminase in a high-speed supernatant of rat liver was more than two orders of magnitude higher than the concentration needed for a similar inhibition of adenosine deaminase in the same preparation (Fig. 4). The inhibitory effect of coformycin on AMP deaminase was not affected by the presence of the physiological inhibitors P_i and GTP. Because of the low enzymic activity in these conditions, the latter study, represented by the dotted line in Fig. 4, had to be performed by a radiochemical method on purified enzyme. It was verified that the addition of 50 μM-coformycin did not influence the activities of other enzymes of the purine-catabolic pathway in crude liver preparations, namely 5'-nucleotidase, nucleoside phosphorylase, xanthine oxidase, guanase and uricase (results not shown).

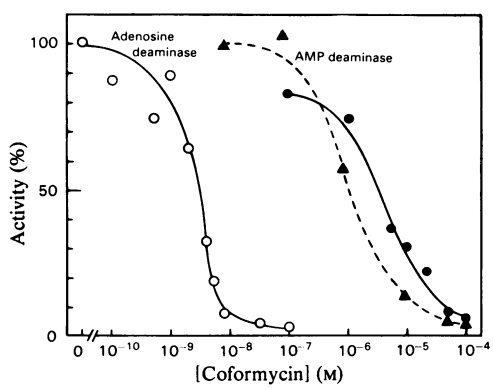

Fig. 4. *Influence of coformycin on the hepatic activities of adenosine deaminase and of AMP deaminase*
The activity of adenosine deaminase (○) was measured in 0.05 ml of a high-speed supernatant at 0.5 mM-adenosine and a reaction velocity of 7.7 nmol/min was measured in the absence of coformycin. The activity of AMP deaminase in the presence of 0.5 mM-AMP and 1 mM-ATP (●) was measured in 0.1 ml of the same preparation, and the 100% value reached 26.7 nmol/min. For the assay of AMP deaminase in the presence of 0.2 mM-AMP, 3 mM-ATP, 5 mM-P_i and 0.2 mM-GTP (▲), 0.05 ml of partially purified enzyme were used and the reaction velocity measured in the absence of coformycin was 0.8 nmol/min.

Effects of coformycin on isolated hepatocytes

On the basal production of allantoin. It was repeatedly verified that the addition of 0.1 μM-coformycin to suspensions of isolated rat hepatocytes did not influence the production of unlabelled as well as of radioactive allantoin. In contrast, the addition of the inhibitor at a concentration of 50 μM inhibited completely the formation of radioactive allantoin, whereas the rate of production of the unlabelled product was decreased by 85% (Fig. 5). Less marked inhibitory effects were recorded after addition of 2 μM-coformycin, but again inhibition of the formation of radioactive allantoin was about 20% greater than that of unlabelled allantoin. The 15 min latency seen in the effect of coformycin may be explained by the time interval required for both its complete penetration inside the hepatocytes and its association with the target enzyme. The decrease in the production of allantoin was accompanied by a slower decline of the radioactivity of the adenine nucleotides (results not shown).

On the fructose-induced breakdown of adenine nucleotides. Fig. 6 shows the effect of the addition of

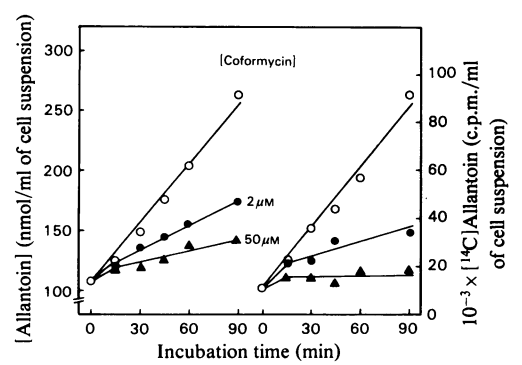

Fig. 5. *Influence of coformycin on the basal production of allantoin by isolated hepatocytes*
After 15 min preincubation, in the presence of 1 μM-[^{14}C]adenine, the hepatocytes were incubated without or with coformycin at the concentrations indicated, and processed as described in the Materials and Methods section.

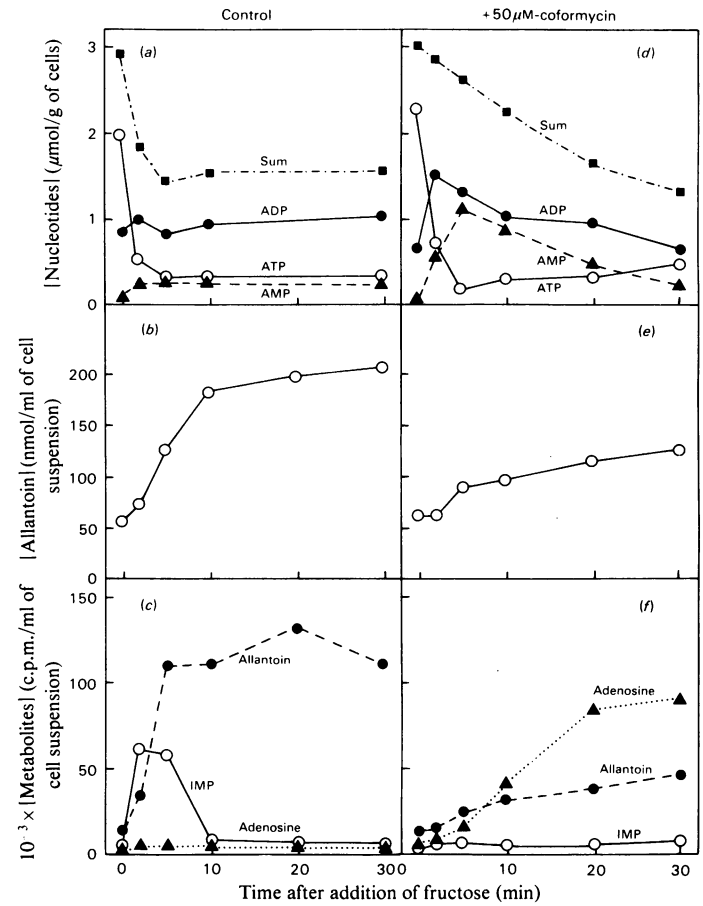

Fig. 6. *Influence of 50μM-coformycin on the fructose-induced degradation of the adenine nucleotides in isolated hepatocytes*

Fructose (1 mg/ml) was added at zero time to hepatocytes that had been preincubated in the absence (*a–c*) or in the presence of 50μM-coformycin (*d–f*). Further manipulations were performed as described in the Materials and Methods section.

fructose on the intracellular concentration of adenine nucleotides, the production of allantoin and the formation of radioactive catabolites in suspensions of isolated rat hepatocytes. The influence of fructose on control hepatocytes, illustrated in Figs. 6(*a*)–6(*c*), is compared with the effect of the ketose on cells that had been preincubated with 50μM-coformycin (Figs. 6*d*–6*f*). The rapid degradation of ATP (Figs. 6*a* and 6*d*) as well as the accumulation of fructose 1-phosphate (not shown) were not modified by the addition of coformycin. The inhibitor provoked, however, a marked elevation in the concentration of AMP, which increased 14-fold above the value at 0min, and to a lesser degree of ADP. As a consequence, depletion of the adenine nucleotide pool proceeded much more slowly, a finding that was reflected in a nearly complete suppression of the fructose-induced burst of the production of allantoin (Figs. 6*b* and 6*e*). As reported by Smith *et al.* (1977*b*), prelabelling of the adenine nucleotide pool of the isolated hepatocytes with [^{14}C]adenine allowed the measurement of the formation of radioactive IMP and allantoin after the addition of fructose, whereas no adenosine could be detected (Figs. 6*c* and 6*f*). Determination of the radioactivity in these catabolites of the adenine nucleotides in the presence of 50μM-coformycin demonstrated its near-absence in IMP, a marked decrease in allantoin and a striking appearance in adenosine. In the presence of 0.1μM-coformycin, the fructose effect was not modified and labelled adenosine was not detected (results not shown).

Discussion

Purine catabolism in isolated hepatocytes

The observation that in isolated rat hepatocytes,

incubated with labelled adenine, 30% of the radioactivity incorporated in the adenine nucleotides was lost after 1h (Fig. 2) is in marked contrast with data obtained with mouse liver *in vivo*, where the half-life of the precursor in the adenine nucleotide pool reached 17–18h (Bennett & Krueckel, 1955). The radioactivity lost from the adenine nucleotides was completely recovered in the allantoin produced by the isolated hepatocytes. It can thus be concluded that the rate of production of allantoin, reaching 32nmol/min per g of cells (Fig. 1), is increased severalfold as compared with the physiological value *in vivo*. A likely explanation for this greatly enhanced catabolism may be the decrease in the concentration of GTP from around 0.5mM in the liver *in vivo* (Clifford *et al.*, 1972; Van den Berghe *et al.*, 1977*a*) to 0.2mM in isolated hepatocytes (Fig. 3*a*). Indeed, GTP constitutes the major inhibitor of hepatic AMP deaminase (Setlow *et al.*, 1966; Smith & Kizer, 1969) and, in conjunction with P_i, has been postulated to account for the physiological inhibition of the enzyme (Van den Berghe *et al.*, 1977*a*). From the latter studies, performed with partially purified enzyme, a decrease in the concentration of GTP from 0.5 to 0.2mM would be expected to increase the rate of production of allantoin severalfold. The reason for the decrease in the concentration of GTP in isolated hepatocytes as compared with the liver *in vivo* is not immediately apparent. It may be caused by transient hypoxia during preparation of the isolated hepatocytes; whereas normal concentrations of ATP are restored during subsequent incubation, the re-formation of GTP is possibly a much slower process. Indeed, studies by Zahn *et al.* (1969) have shown that the hepatic transfer of P_i to ADP, via oxidative and substrate phosphorylation, occurs several-fold faster than the transfer of P_i from ATP to GDP. The finding that the rate of production of allantoin by isolated hepatocytes could be decreased by increasing the concentration of P_i is in keeping with the known inhibitory effect of the latter anion on hepatic AMP deaminase (Nikiforuk & Colowick, 1956; Van den Berghe *et al.*, 1977*a*). Whereas Smith *et al.* (1977*a*,*b*) added 10–100μM-[^{14}C]adenine to the isolated hepatocytes in order to label the adenine nucleotide pool, we have used the precursor at the tracer concentration of 1μM. This modification offers the advantage that incorporation of the label is complete within 2min. Washing of the hepatocytes after the preincubation period was therefore not required. Incorporation was followed by a linear decrease of the radioactivity in the adenine nucleotides, accompanied by a closely equivalent appearance of the label in allantoin. The finding that the other purine nucleotides were not labelled to any significant extent denotes that the measurement of the radioactivity in allantoin constitutes a valid indication of the breakdown of the adenine nucleotides.

Influence of coformycin on purine catabolism in isolated hepatocytes

From our enzyme studies, it appears that whereas hepatic adenosine deaminase can be maximally inhibited by 0.1μM-coformycin, AMP deaminase requires 50μM of the nucleoside antibiotic. The lower sensitivity of the latter enzyme is in agreement with the data obtained by Agarwal & Parks (1977) with muscle enzyme. We have observed furthermore that the inhibitory effect of coformycin on the ATP-stimulated AMP deaminase was not modified by the addition of its physiological inhibitors. Since none of the other hepatic enzymes of purine catabolism was influenced by the maximal concentrations of coformycin used in our experiments with isolated hepatocytes, it can be reasonably assumed that only the deaminations of adenosine and of AMP were affected in this system.

The observation that the basal production of unlabelled as well as of radioactive allantoin was not influenced by a concentration of coformycin that maximally inhibited adenosine deaminase in a liver extract constitutes a strong indication that the catabolism of the adenine nucleotides does not occur through this enzyme. The good quantitative agreement between the inhibitory effects of two concentrations of coformycin on the activity of hepatic AMP deaminase and on the production of allantoin by isolated hepatocytes provides, in view of the complete penetration of the inhibitor, suggestive evidence that the latter enzyme constitutes the rate-limiting step in the catabolism of the adenine nucleotides in the liver. The incomplete inhibition by 50μM-coformycin of the basal production of unlabelled, as compared with radioactive, allantoin is most probably explained by the fact that the inhibitor does not affect the catabolism of other purines, mainly guanine derivatives.

These conclusions are reinforced by the analysis of the influence of coformycin on the fructose-induced catabolism of the adenine nucleotides. Coformycin at the concentration of 0.1μM did not modify the effect of fructose observed in the absence of the inhibitor, whereas drastic changes became apparent in the presence of 50μM-coformycin. Under these conditions, the depletion of ATP as well as the accumulation of fructose 1-phosphate induced by the addition of fructose were not modified, indicating that the metabolism of the ketose was not affected. Striking elevations in the concentrations of ADP and AMP, the latter increasing 14-fold above the basal value, were, however, observed in the presence of the inhibitor as compared with the control experiment. Moreover, the transient accumulation of IMP observed after the administration of a

fructose load (Woods *et al.*, 1970; Van den Berghe *et al.*, 1977*a*) was not recorded any more, indicating inhibition of AMP deaminase. In contrast, adenosine, the presence of which is normally not detectable, was now formed in large amounts, most probably through the hydrolysis of AMP by the soluble 5′-nucleotidase. Indeed, because of its sigmoidal kinetics (Van den Berghe *et al.*, 1977*b*) the reaction does not occur at low and physiological concentration of AMP, but occurs well in the presence of the higher concentration reached in the presence of fructose plus coformycin. In accordance with this explanation is the finding that the formation of adenosine follows the build-up of AMP. The finding that the formation of allantoin, although delayed and much smaller than in the control experiment, remained measurable, may be explained by a still incomplete inhibition of AMP deaminase at 50μM-coformycin (cf. Fig. 4).

The experiments reported constitute additional evidence to define AMP deaminase as the limiting step in the catabolism of the hepatic adenine nucleotides. They provide a further stimulus to search for abnormalities of this enzyme or of its regulation in human subjects in order to explain the pathogenesis of primary gout (Hers & Van den Berghe, 1979).

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