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The pathway of adenine nucleotide catabolism and its control in isolated rat hepatocytes subjected to anoxia

Marie-Françoise VINCENT, Georges VAN DEN BERGHE 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 breakdown of the adenine nucleotide pool provoked by the replacement of the O_2/CO_2 gas phase by N_2/CO_2 was studied in isolated rat hepatocytes with the purpose of defining the pathway of the catabolism of AMP in anoxic conditions. 2. Approx. 40% of the adenine nucleotide pool was lost after 40–60 min of anoxia. In hepatocytes from fed rats there was a slow disappearance of ATP. This is explained by the presence of glycogen stores, allowing the generation of ATP by anaerobic glycolysis. In hepatocytes from 24 h-starved rats, ATP almost completely disappeared within 5 min, and was partly replaced by an accumulation of AMP. This indicates that another mechanism protects the adenine nucleotide pool in the starved state. In both conditions, the loss of adenine nucleotides was mainly accounted for by an accumulation of uric acid, owing to the oxygen-dependence of urate oxidase. 3. Incubation of the hepatocytes before the suppression of O_2 with coformycin at concentrations known to inhibit selectively adenosine deaminase did not result in an accumulation of adenosine and did not influence the formation of uric acid. This indicates that the degradation of AMP does not proceed by way of 5′-nucleotidase under these conditions. In the presence of coformycin at concentrations which are inhibitory to AMP deaminase, however, the formation of uric acid was nearly suppressed, demonstrating that the initial degradation of AMP was catalysed by the latter enzyme. 4. The accumulation of AMP in the starved state can be explained by the pronounced decrease in ATP, the major stimulator of AMP deaminase, and the enhanced increase in P_i, one of its physiological inhibitors. The modifications of these effectors can also explain the increased inhibition of the cytoplasmic 5′-nucleotidase, shown by the accumulation of IMP in the absence of coformycin, in hepatocytes from starved rats. 5. Reoxygenation of the hepatocytes after 20 min of anoxia induced a prompt regeneration of ATP, which reached concentrations equal to the pre-existing concentration of AMP. 6. No explanation was found for the accumulation of IMP observed after preincubation of the hepatocytes with 0.1 μM-coformycin, since the activities of the IMP-metabolizing enzymes were not influenced by this inosine analogue.

From previous work with partially purified enzymes (Van den Berghe *et al.*, 1977*a*,*b*) we have come to the conclusion that the degradation of physiological concentrations of AMP (0.1–0.2 mM) proceeds exclusively by deamination to IMP in the liver. AMP deaminase is normally 95% inhibited at physiological concentrations of its substrate, activator (ATP), and inhibitors (GTP and P_i). A dephosphorylation of AMP was ruled out, since non-cytosolic 5′-nucleotidases do not qualify for a role in purine catabolism and because the cytoplasmic 5′-nucleotidase displays sigmoidal kinetics that prevent the dephosphorylation of physiological concentrations of AMP. These conclusions, as well as the proposal that AMP deaminase constitutes the limiting step of purine catabolism in physiological conditions, were confirmed by experiments in isolated hepatocytes (Van den Berghe *et al.*, 1980).

The possibility remained, nevertheless, that the cytoplasmic 5′-nucleotidase may become active on AMP at markedly higher concentrations of this nucleotide, such as those observed in anoxic conditions (Deuticke & Gerlach, 1966; Busch *et al.*, 1968; Brosnan *et al.*, 1970; Hems & Brosnan, 1970; Jackson *et al.*, 1976; Sharma *et al.*, 1980). Besides a loss of ATP, a decrease in the concentration of GTP

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(Deuticke & Gerlach, 1966; Jackson *et al.*, 1976) and an increase in the concentration of P_i (Hems & Brosnan, 1970) have also been reported in this situation.

The present work was performed to test the possibility that hepatic AMP may be dephosphorylated to adenosine in anoxic conditions as well as to assess the role of the regulation of the cytoplasmic 5'-nucleotidase by ATP, GTP and P_i (Van den Berghe *et al.*, 1977*b*; Itoh *et al.*, 1978). Anoxia was induced by the replacement of O_2 by N_2 in the incubation medium of isolated rat liver cells. Hepatocytes prepared from starved rats were compared with those isolated from well-fed animals, in which anaerobic formation of ATP was sustained by glycolysis from glycogen. As in our previous work (Van den Berghe *et al.*, 1980), the nucleoside antibiotic coformycin was used in order to determine the pathway of degradation of AMP. Indeed, low concentrations of this inosine analogue inhibit adenosine deaminase in various tissues (Sawa *et al.*, 1967; Snyder & Henderson, 1973; Agarwal *et al.*, 1975), including the liver, in which complete inhibition was observed with 0.1 μM inhibitor (Van den Berghe *et al.*, 1980). This inhibition would therefore result in an accumulation of adenosine only if the initial degradation of AMP occurs by way of 5'-nucleotidase. Higher concentrations of coformycin also inhibit AMP deaminase in muscle (Agarwal & Parks, 1977) and in the liver, in which maximal inhibition is reached with concentrations around 50 μM (Van den Berghe *et al.*, 1980).

Materials and methods

Chemicals and enzymes

Coformycin was a gift from Professor H. Umezawa (Institute of Microbial Chemistry, Tokyo, Japan). Cytoplasmic 5'-nucleotidase of rat liver was partially purified as described previously (Van den Berghe *et al.*, 1977*b*). The source of all other chemicals and enzymes has been given (Van den Berghe *et al.*, 1980).

Experiments with isolated hepatocytes

Hepatocytes were prepared as previously described (Van den Berghe *et al.*, 1980), except that starved (24h) as well as fed male Wistar rats were used and that no glucose was added to the incubation medium in the former condition. Samples contained about 60mg of cells/ml. Experiments shown are representative of three to four repeat observations. The isolated cells were preincubated first with 1 μM-[^{14}C]adenine for 5min in order to label the adenine nucleotide pool. This was followed by a second, 15min, preincubation in order to allow coformycin, when added, to penetrate completely inside the hepatocytes and to associate with its target enzymes (Van den Berghe *et al.*, 1980).

Anoxia was induced by the replacement of the O_2/CO_2 (19:1) gas phase by identical proportions of N_2/CO_2. The methods used for the spectrophotometric determination and the t.l.c. separation of nucleotides, nucleosides and their breakdown products have been given (Van den Berghe *et al.*, 1980). The concentrations of IMP and adenosine were calculated from the radioactivity appearing in these metabolites. Preliminary experiments, in which IMP was determined by high-pressure anion-exchange chromatography (Hartwick & Brown, 1975) and adenosine by the method of Kalckar (1947), had shown that their specific radioactivity was identical with and evolved in parallel with that of the adenine nucleotides.

GTP was also measured by high-pressure anion-exchange chromatography. For the determination of the intracellular concentration of phosphate, samples of the cell suspension were poured into the separation tubes designed by Hems *et al.* (1975). Phosphate was determinated on the $HClO_4$ extracts by the method of Fiske & SubbaRow (1925).

Measurement of enzyme activities

The activity of the partially purified cytoplasmic 5'-nucleotidase was measured as previously described (Van den Berghe *et al.*, 1977*b*), except that the separation of radioactive substrate and product was performed as described by Reibel & Rovetto (1978). Adenylosuccinate synthetase was measured as described by Bogusky *et al.* (1976) and IMP dehydrogenase as described by Wu & Scrimgeour (1973). High-speed supernatants of rat liver, prepared by centrifuging 20% (w/v) homogenates in 90mM-potassium phosphate buffer (pH7)/180mM-KCl at 100000*g* for 60min, were used after filtration on Sephadex G-25.

Results

Influence of anoxia on the adenine nucleotide pool in hepatocytes from fed rats

The effects of the replacement of the O_2/CO_2 gas mixture by N_2/CO_2 in a representative experiment performed with isolated hepatocytes from well-fed animals are shown in Fig. 1. A slow depletion of ATP was accompanied by a progressive increase in the concentration of AMP, accounting for 20% of the loss of ATP at 40min. The sum of the adenine nucleotides decreased to approx. 60% of its initial value after this time interval. A small, short-lived increase in the concentration of IMP was also observed (Fig. 1*a*), but the concentrations of adenosine (Fig. 1*b*), hypoxanthine and xanthine (results not shown) were not modified. The production of allantoin, which amounted to 31nmol/min

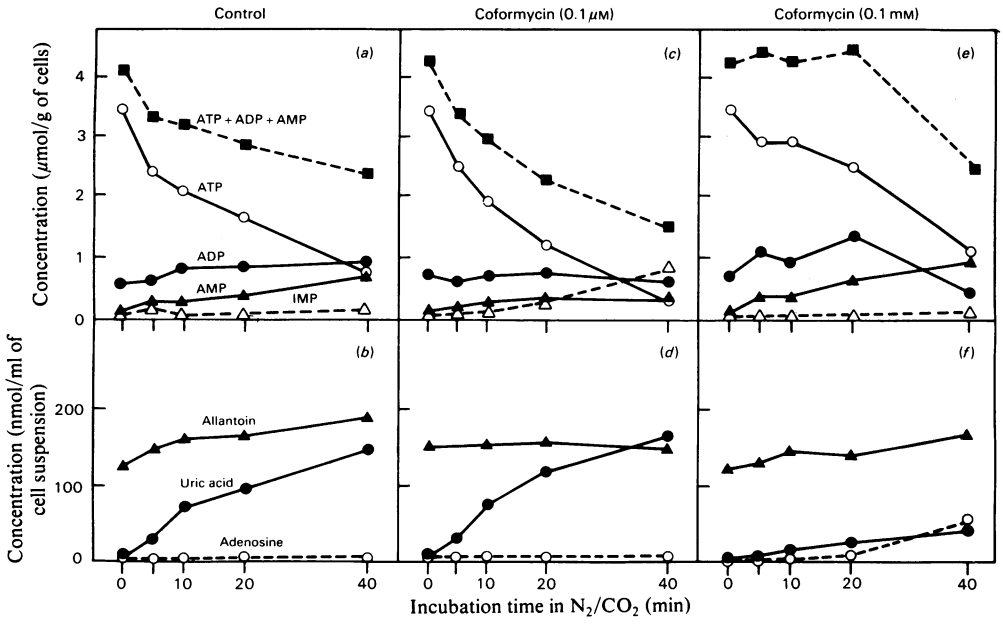

Fig. 1. *Influence of coformycin on the degradation of the adenine nucleotides induced by anoxia in isolated hepatocytes from fed rats*

The O_2/CO_2 gas phase was replaced by N_2/CO_2 at time zero in hepatocyte suspensions that had been preincubated in the absence (*a*, *b*) or presence of coformycin at 0.1 μM (*c*, *d*) or 0.1 mM (*e*, *f*). Further manipulations were performed as described in the Materials and methods section.

per g in control cells incubated simultaneously in the presence of oxygen (results not shown), was barely modified. Uric acid, however, which was not measurable before the induction of anoxia, accumulated at the rate of 106 nmol/min per g of hepatocytes during the first 10 min, and of 40 nmol/min per g thereafter.

The effect of the prior addition of 0.1 μM-coformycin on this catabolism is shown in Figs. 1(*c*) and 1(*d*). A slightly more rapid decrease in the concentration of ATP and of the sum of the adenine nucleotides was accompanied by a progressive accumulation of IMP, whereas the formation of adenosine and the build-up of uric acid were not different from those in the control experiment.

On preincubation with 0.1 mM-coformycin, the adenine nucleotide pool was clearly preserved (Figs. 1*e* and 1*f*). The decrease in the concentration of ATP was markedly slower and accompanied, for 20 min, by an equivalent increase in AMP and ADP, so that the sum of the adenine nucleotides did not change during this time interval. Whereas the accumulation of IMP was nearly completely suppressed under these conditions, a slight increase in the concentration of adenosine was observed. In accordance with the marked inhibition of the breakdown of the adenine nucleotides, only a minimal increase in the concentration of uric acid was recorded, its rate of production reaching only 16 nmol/min per g of cells.

In all experimental conditions, the sum of the concentrations of all the measured nucleotides, nucleosides and their breakdown products increased linearly with time. Over 40 min, the total concentration of purine compounds increased by approx. 1.2 μmol/g of hepatocytes.

Influence of anoxia on the adenine nucleotide pool in hepatocytes from starved rats

In hepatocytes from starved rats, the initial concentration of ATP was usually lower than in the fed state (Fig. 2). Induction of anoxia (Figs. 2*a* and 2*b*) led to a rapid loss of approx. 90% of the ATP within 5 min. This was accompanied by a slightly less rapid accumulation of AMP, accounting for 67% of the loss of ATP. Thereafter, the concentration of AMP remained virtually unchanged for 50 min. The sum of the adenine nucleotides, after an approx. 20% initial decrease, stayed fairly constant during the same time interval. The swift, sustained accumulation of IMP, accounting for 20% of the loss of ATP, is in marked contrast with the results

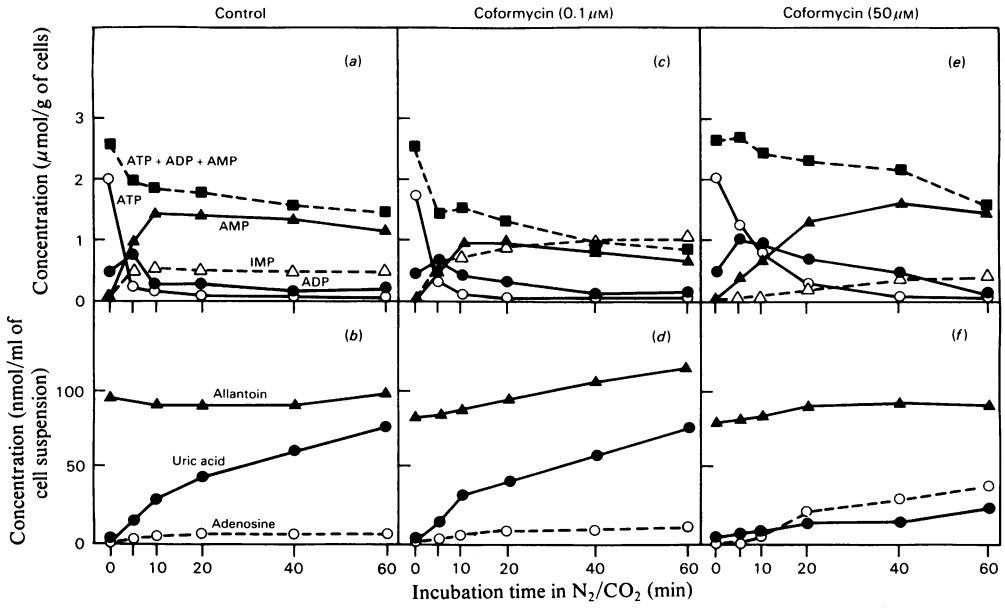

Fig. 2. *Influence of coformycin on the degradation of the adenine nucleotides induced by anoxia in isolated hepatocytes from starved rats*

The experiments were performed as described in the legend to Fig. 1, except that coformycin was used at a concentration of 50 μM instead of 0.1 mM.

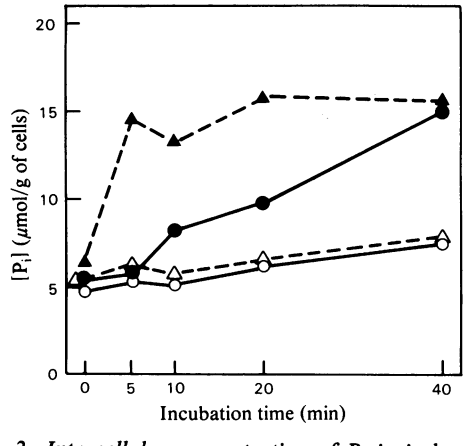

Fig. 3. *Intracellular concentration of P_i in isolated rat hepatocytes*

The concentration of P_i was determined as described in the Materials and methods section in hepatocytes that were isolated from fed (circles) or starved (triangles) rats, and thereafter incubated in O_2/CO_2 (open symbols) or in N_2/CO_2 (closed symbols). Each point represents the mean of two independent experiments.

obtained in the fed state. The concentration of adenosine, although slightly higher than in the fed state, remained negligible. The production of allantoin, which reached 23 nmol/min per g of cells in hepatocytes incubated in oxygen, became minimal and was replaced by a nearly equivalent production of uric acid, amounting to 27 nmol/min per g of cells over 60 min.

As in isolated hepatocytes from fed rats, preincubation with 0.1 μM-coformycin provoked a slightly more rapid degradation of the adenine nucleotide pool (Figs. 2*c* and 2*d*). The acccumulation of IMP was markedly enhanced, but the formation of adenosine, allantoin and uric acid was not significantly modified. In the presence of 50 μM-coformycin (Figs. 2*e* and 2*f*) the decrease in the concentration of ATP as well as the accumulation of AMP proceeded at a significantly slower rate, and the rapid initial decrease in the sum of the adenine nucleotides was suppressed. The accumulation of IMP was also inhibited. The production of uric acid was decreased by 75% to a rate of 7 nmol/min per g of cells, whereas an accumulation of adenosine became evident. The increase in the total amount of all measured purine compounds averaged 1.2 μmol/g of hepatocytes over 60 min in the various experimental conditions.

Influence of anoxia on the intracellular concentration of P_i and GTP

The potential importance of P_i in the regulation of

both AMP deaminase and the cytoplasmic 5'-nucleotidase prompted a study of the influence of anoxia on the intracellular concentration of this metabolite. As shown in Fig. 3, the concentration of P_i increased more rapidly in hepatocytes from starved rats subjected to anoxia than in cells isolated from fed animals. Measurements of the concentration of GTP in the isolated hepatocytes revealed that it decreased, in parallel with the concentration of ATP, from a control value of approx. 0.2 μmol/g of cells in both conditions, to nearly undetectable values in the starved state (results not shown).

Recovery of ATP on reoxygenation

The reversibility of the modifications induced by anoxia was investigated by replacing the N_2/CO_2 mixture again by O_2/CO_2 after a certain time interval. Fig. 4 depicts an experiment performed with hepatocytes from starved rats. In the absence as well as in the presence of coformycin, the reoxygenation of the hepatocytes after 20 min of anoxia induced a prompt accumulation of ATP, accompanied by an equally rapid decrease in AMP. Within 2 min after the introduction of oxygen, the concentration of ATP reached in the different experimental conditions was equal to the pre-existing concentration of AMP. This resulted in a complete recovery of the initial concentration of ATP in the cells that had been preincubated with 0.1 mM-coformycin, in which little degradation of AMP to IMP had occurred. Reoxygenation was also followed by a decrease in the concentration of IMP, which proceeded at approximately the same rate in all three experimental conditions, but was markedly slower than the recovery of ATP and the fall of AMP. The build-up of uric acid observed in the absence and in the presence of 0.1 μM-coformycin during anoxia was rapidly replaced by an accumulation of allantoin on reoxygenation (results not shown).

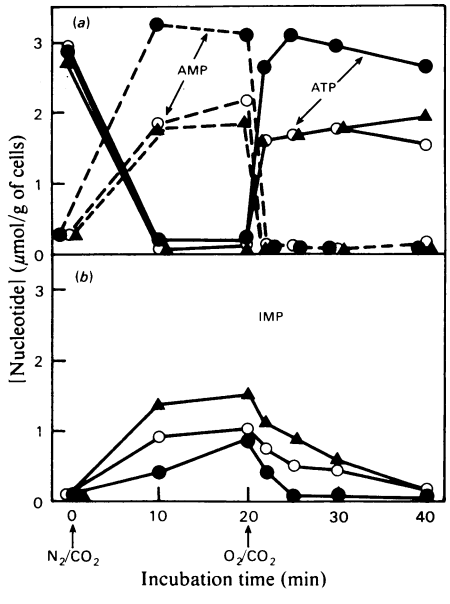

Fig. 4. *Influence of anoxia followed by reoxygenation on the concentrations of (a) ATP (——) and AMP (– – – –) and (b) IMP in isolated hepatocytes*

The O_2/CO_2 gas phase was replaced at zero time by N_2/CO_2 in hepatocyte suspensions from starved rats that had been preincubated in the absence of coformycin (○) or in the presence of coformycin at 0.1 μM (▲) or 0.1 mM (●). O_2/CO_2 was reintroduced instead of N_2/CO_2 at 20 min. Further manipulations were performed as described in the Materials and methods section.

Influence of coformycin on the IMP-metabolizing enzymes

In order to find an explanation for the enhancement of the accumulation of IMP observed in the presence of 0.1 μM-coformycin (Figs. 1*c*, 2*c* and 4*b*) the influence of the nucleoside antibiotic on the activity of the IMP-metabolizing enzymes was investigated. Concentrations of coformycin up to 10 μM did not influence the activities of the cytoplasmic 5'-nucleotidase, of adenylosuccinate synthetase and of IMP dehydrogenase in rat liver extracts (results not shown).

Discussion

A dual mechanism for the preservation of the adenine nucleotide pool in anoxic hepatocytes

In isolated rat hepatocytes incubated in N_2/CO_2, the decrease in the concentration of ATP was accompanied by an increase in that of AMP; approx. 60% of the adenine nucleotide pool was still preserved after 40–60 min. The restricted degradation of the adenine nucleotides is also apparent from the limited increase in the rate of production of their breakdown products. In hepatocytes from fed rats, this rate was increased 4-fold during 10 min and less than 2-fold thereafter. In the starved state, the rate of uric acid formation did not significantly exceed that of allantoin in aerobic conditions. The production of uric acid instead of allantoin in these conditions is explained by the oxygen-dependence of urate oxidase (Keilin & Hartree, 1936). It is also confirmatory of the hypothesis that xanthine oxidase is in the dehydrogenase (type D) form intracellularly (Stirpe & Della Corte, 1969).

In hepatocytes from fed animals, the preservation of the adenine nucleotide pool was mainly due to a

better maintenance of the ATP content as compared with the starved state. This observation, as well as the usually higher initial concentration of ATP in hepatocytes from fed rats, is in agreement with data obtained in intact livers subjected to ischaemia (Hems & Brosnan, 1970; Sharma *et al.*, 1980). Both findings may be explained by the presence of glycogen stores, allowing the generation of ATP by glycolysis during the isolation procedure as well as during the incubation in the presence of N_2. In hepatocytes from starved animals, in which as much as 90% of the ATP content disappeared within 5 min of anoxia, the preservation of the adenine nucleotide pool was due to a rapid and marked increase in the concentration of AMP. This indicates that another mechanism operates in the absence of anaerobic glycolysis caused by the lack of glycogen.

Purine synthesis *de novo* may also play a role in the preservation of the adenine nucleotide pool, as indicated by the progressive increase in the total amount of purine compounds in the isolated liver cells and in their incubation medium. However, this mechanism did not seem influenced by anoxia or by the nutritional status of the hepatocytes.

The pathway of AMP degradation and its control

The predominant role of AMP deaminase in the degradation of AMP in anoxia is indicated by: (1) the accumulation of IMP in the hepatocytes from starved rats; (2) the fact that this accumulation as well as the formation of uric acid were inhibited by coformycin at concentrations at which it is inhibitory of AMP deaminase; (3) the absence of accumulation of adenosine in the presence of a concentration of coformycin that inhibits selectively adenosine deaminase.

The preservation of the hepatic adenine nucleotide pool in anoxic conditions indicates that potent control mechanisms restrict the activities of both AMP deaminase and 5′-nucleotidase, despite the large increase in AMP. The limited degradation of AMP by AMP deaminase may be explained by the decrease in the concentration of ATP, the major stimulator of the liver enzyme, and by the increase in P_i, one of its physiological inhibitors (Van den Berghe *et al.*, 1977*a*). The higher build-up and slower breakdown of AMP in the starved as compared with the fed state can be accounted for by the more pronounced depletion of ATP and enhanced accumulation of P_i in hepatocytes from starved rats. The latter observations are in accordance with the findings of Hems & Brosnan (1970) in the liver *in vivo*. Calculation revealed that, in the experiments described by these authors, the increase in the intracellular concentration of P_i matched the loss of phosphate from the adenine nucleotides. In our studies, however, the increase in the intracellular P_i was higher than could be accounted for by the degradation of the adenine nucleotides. This suggests a release of P_i from other phosphate esters or an enhancement of the entry of this ion into the hepatocytes. Still another factor explaining the nearly complete arrest of the degradation of AMP observed for as long as 1 h in hepatocytes from starved rats may be accumulation of IMP and resulting feed-back inhibition of AMP deaminase (Van den Berghe *et al.*, 1977*a*).

The negligible participation of the cytoplasmic 5′-nucleotidase in the initial degradation of AMP can be explained by the kinetic properties of the enzyme (Van den Berghe *et al.*, 1977*b*). Indeed, the increase in activity that may have been expected from the elevation of the substrate concentration has apparently been counteracted by the decrease in the concentration of the stimulators of the enzyme, ATP and GTP, and by the increase in the concentration of its inhibitor, P_i. This interpretation is strengthened by the observation that IMP accumulates in anoxic hepatocytes from starved rats incubated without coformycin, indicating that the function of the 5′-nucleotidase in the second degradation step of AMP is also impaired in these conditions. This may be accounted for by the more pronounced decrease in ATP and GTP and the enhanced accumulation of P_i in the starved state, and explains the low rate of degradation of the adenine nucleotides in this situation. The accumulation of adenosine observed in the presence of coformycin at concentrations that inhibit AMP deaminase might be explained by a decrease of the competitive inhibition exerted by IMP on the hydrolysis of AMP (Van den Berghe *et al.*, 1977*b*).

The experiments reported in the present paper thus confirm the proposal by Deuticke & Gerlach (1966) that the initial degradation of hepatic AMP in anoxic conditions occurs by way of AMP deaminase, rather than by dephosphorylation as asserted by Busch *et al.* (1968). They further substantiate our previous conclusions that the regulation of the liver cytoplasmic 5′-nucleotidase is uniquely designed to preserve the adenine nucleotide pool in this tissue (Van den Berghe *et al.*, 1977*b*) and that AMP deaminase constitutes the limiting step in the catabolism of the adenine nucleotides (Van den Berghe *et al.*, 1977*a*, 1980). This work also confirms our previous assertion that the non-cytosolic 5′-nucleotidases, the membranous enzyme in particular, do not participate in hepatic purine catabolism.

Pathways of IMP metabolism

The reoxygenation experiments show that AMP can be completely re-converted into ATP, presumably by rephosphorylation in the mitochondria. This indicates that the hepatocytes have maintained a satisfactory functional integrity. The ob-

servation that IMP was not re-converted into adenine nucleotides indicates that the enzymic sequence adenylosuccinate synthetase–adenylosuccinate lyase is not operative under these conditions. This may be explained by the loss of GTP, which is required for the synthesis of adenylosuccinate. The progressive decreases in the concentration of IMP recorded in the absence as well as in the presence of coformycin on re-addition of oxygen can be explained by an enhancement of the activity of the cytoplasmic 5'-nucleotidase, caused by the increase in ATP in conjunction with a decrease in the concentration of P_i. We have no explanation as yet for the augmenting effect of 0.1 μM-coformycin on the accumulation of IMP. Indeed, the nucleoside antibiotic did not inhibit the cytoplasmic 5'-nucleotidase, nor the two alternative enzymes of IMP metabolism, adenylosuccinate synthetase and IMP dehydrogenase. The possibility remains that the inhibitory effect may be exerted by a product of the metabolism of coformycin in the hepatocytes, e.g. a phosphorylated derivative (Frieden *et al.*, 1979).

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References

Agarwal, R. P. & Parks, R. E., Jr. (1977) *Biochem. Pharmacol.* **26**, 663–666.

Agarwal, R. P., Sagar, S. M. & Parks, R. E., Jr. (1975) *Biochem. Pharmacol.* **24**, 693–701

Bogusky, R. T., Lowenstein, L. M. & Lowenstein, J. M. (1976) *J. Clin. Invest.* **58**, 326–335

Brosnan, J. T., Krebs, H. A. & Williamson, D. H. (1970) *Biochem. J.* **117**, 91–96

Busch, E. W., Von Borcke, I. M. & Martinez, B. (1968) *Biochim. Biophys. Acta* **166**, 547–556

Deuticke, B. & Gerlach, E. (1966) *Pflügers Arch.* **292**, 239–254

Fiske, C. H. & SubbaRow, Y. (1925) *J. Biol. Chem.* **66**, 375–400

Frieden, C., Gilbert, H. R., Miller, W. H. & Miller, R. L. (1979) *Biochem. Biophys. Res. Commun.* **91**, 278–283

Hartwick, R. A. & Brown, P. R. (1975) *J. Chromatogr.* **112**, 651–662

Hems, D. A. & Brosnan, J. T. (1970) *Biochem. J.* **120**, 105–111

Hems, R., Lund, P. & Krebs, H. A. (1975) *Biochem. J.* **150**, 47–50

Itoh, R., Usami, C., Nishino, T. & Tsushima, K. (1978) *Biochim. Biophys. Acta* **526**, 154–162

Jackson, R. C., Boritzki, T. J., Morris, H. P. & Weber, G. (1976) *Life Sci.* **19**, 1531–1536

Kalckar, H. M. (1947) *J. Biol. Chem.* **167**, 445–459

Keilin, D. & Hartree, E. F. (1936) *Proc. R. Soc. London Ser. B* **119**, 114–140

Reibel, D. K. & Rovetto, M. J. (1978) *J. Chromatogr.* **161**, 406–409

Sawa, T., Fukagawa, Y., Homma, H., Takeuchi, T. & Umezawa, H. (1967) *J. Antibiot. Ser. A* **20**, 227–231

Sharma, R. J., Rodrigues, L. M., Whitton, P. D. & Hems, D. A. (1980) *Biochim. Biophys. Acta* **630**, 414–424

Snyder, F. F. & Henderson, J. F. (1973) *J. Biol. Chem.* **248**, 5899–5904

Stirpe, F. & Della Corte, E. (1969) *J. Biol. Chem.* **244**, 3855–3863

Van den Berghe, G., Bronfman, M., Vanneste, R. & Hers, H. G. (1977*a*) *Biochem. J.* **162**, 601–609

Van den Berghe, G., Van Pottelsberghe, C. & Hers, H. G. (1977*b*) *Biochem. J.* **162**, 611–616

Van den Berghe, G., Bontemps, F. & Hers, H. G. (1980) *Biochem. J.* **188**, 913–920

Wu, T. W. & Scrimgeour, K. G. (1973) *Can. J. Biochem.* **51**, 1380–1390