Adenine nucleotide metabolism in isolated chicken hepatocytes

Józef SPYCHAŁA* and Georges VAN DEN BERGHE†

Laboratory of Physiological Chemistry, UCL and International Institute of Cellular and Molecular Pathology, Avenue Hippocrate 75, B-1200 Brussels, Belgium

The turnover of the adenine nucleotide pool, the pathway of the degradation of AMP and the occurrence of recycling of adenosine were investigated in isolated chicken hepatocytes, in which the adenylates had been labelled by prior incubation with [¹⁴C]adenine. Under physiological conditions, 85% of the IMP synthesized by the 'de novo' pathway (approx. 37 nmol/min per g of cells) was catabolized directly via inosine into uric acid, and 14% was converted into adenine nucleotides. The latter were found to turn over at the rate of approx. 5 nmol/min per g of tissue. Inhibition of adenosine deaminase by 1 μM-coformycin had no effect on the formation of labelled uric acid, indicating that the initial degradation of AMP proceeds by way of deamination rather than dephosphorylation. Inhibition of adenosine kinase by 100 μM-5-iodotubercidin resulted in a loss of labelled ATP, demonstrating that adenosine is normally formed from AMP but is recycled. Unexpectedly, 5-iodotubercidin did not decrease the total concentration of ATP, indicating that the loss of adenylates caused by inhibition of adenosine kinase was nearly completely compensated by formation of AMP *de novo*. Anoxia induced a greatly increased catabolism of the adenine nucleotide pool, which proceeded in part by dephosphorylation of AMP. On reoxygenation, the formation of AMP *de novo* was increased 8-fold as compared with normoxic conditions. The latter results indicate the existence of adaptive mechanisms in chick liver allowing, when required, channelling of the metabolic flux through the 'de novo' pathway, away from the uricotelic catabolic route, into the synthesis of adenine nucleotides.

INTRODUCTION

In ureotelic animals, the synthesis of purines *de novo* (step 1 in Scheme 1) serves to provide adenylates and guanylates, whereas in uricotelic species it constitutes in addition the pathway leading to the excretion of excess amino nitrogen in the form of uric acid. Accordingly, a 15-fold higher rate of purine synthesis *de novo* was measured in chick as compared with rat liver slices (Lipstein *et al.*, 1978). The necessity to preserve the high-energy phosphates, of which ATP, maintained in equilibrium with ADP and AMP by adenylate kinase (step 2), is the main component, and the dual function of the 'de novo' pathway in uricotelic animals, raise the question of the turnover of their hepatic adenine nucleotide pool. Indeed, the adenylates could either be continuously synthesized and degraded at the same rate as the synthesis *de novo*, or have a slower turnover because of a functional separation from this pathway. The latter situation requires strict limitation both of the conversion of IMP into AMP, catalysed by the sequential action of adenylosuccinate synthetase (step 3) and adenylosuccinate lyase (step 4), and of the degradation of AMP. The control of the degradation of AMP is complicated by the fact that its initial steps can occur by two different pathways: either a prior deamination by AMP deaminase (step 5), followed by dephosphorylation of IMP by the cytoplasmic 5'-nucleotidase (step 6), or a prior dephosphorylation by the latter enzyme, followed by deamination of adenosine by adenosine deaminase (step 7). In addition, the

Scheme 1. Pathways of formation and degradation of adenylates in the avian liver

(1) Purine synthesis *de novo*; (2) adenylate kinase; (3) adenylosuccinate synthetase; (4) adenylosuccinate lyase; (5) AMP deaminase; (6) cytoplasmic 5'-nucleotidase; (7) adenosine deaminase; (8) adenosine kinase; (9) nucleoside phosphorylase; (10) xanthine oxidase. Abbreviations: Ado, adenosine; AS, adenylosuccinate; Asp, aspartate; FUM, fumarate; HX, hypoxanthine; Ino, inosine; PRPP, 5-phosphoribosyl 1-pyrophosphate.

* Permanent address: Department of Biochemistry, Academic Medical School, ul. Debinki 1, 80-211 Gdansk, Poland.

† To whom reprint requests should be addressed.

formation of adenosine may not contribute to the production of uric acid, because of recycling by adenosine kinase (step 8).

In the present work, the turnover of the adenine nucleotide pool and the pathway of degradation of AMP have been investigated in isolated chicken hepatocytes both under physiological conditions and in anoxia. The cells were incubated with [^{14}C]adenine in order to label their adenylates, and the concentration of total and of labelled purine compounds was measured at various time intervals. As in our previous studies (Van den Berghe *et al.*, 1980; Vincent *et al.*, 1982; Bontemps *et al.*, 1983, 1986), inhibition of adenosine deaminase by coformycin at low concentration, of AMP deaminase by coformycin at high concentration and of adenosine kinase by 5-iodotubercidin was applied to evaluate the contribution of these enzymes to the pathway and to the control of the degradation of AMP.

We show that the turnover of the adenine nucleotide pool in isolated chicken hepatocytes, incubated under physiological conditions, is markedly slower than the rate of purine biosynthesis *de novo*, indicating strict control of the synthesis and of the catabolism of the adenylates. We further demonstrate that the degradation of AMP leading to the production of uric acid proceeds only by way of IMP under physiological conditions, but also by way of adenosine in anoxia. Finally, we give evidence that the formation of AMP from IMP provided by the '*de novo*' pathway can be modulated according to the necessity to maintain the adenine nucleotide pool.

MATERIALS AND METHODS

Chemicals and enzymes

Coformycin {(R)-3-(β -D-erythropentofuranosyl)-3,6,7,8-tetrahydroimidazol[4,5-d][1,3]diazepin-8-ol} was given by Dr. H. Umezawa (Institute of Microbial Chemistry, Tokyo, Japan). 5-Iodotubercidin {4-amino-5-iodo-7-(β -D-ribofuranosyl[2,3-d]pyrimidine} was provided by Dr. L. B. Townsend (University of Michigan College of Pharmacy, Ann Arbor, MI, U.S.A.). [^{14}C]Adenine (310 Ci/mol), [^{14}C]adenosine (500 Ci/mol) and [8- ^{14}C]hypoxanthine (53 Ci/mol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. For the measurement of adenosine deaminase and of AMP deaminase in crude chicken liver extracts, high-speed supernatants were obtained by centrifuging 20% (w/v) homogenates prepared in 50 mM-imidazole buffer (pH 6.5)/0.1 M-KCl at 100 000 g for 60 min. Partially purified AMP deaminase from chicken liver was prepared as described by Spychala & Makarewicz (1983), except that the enzyme was eluted with 2 M-KCl, pH 7.0, instead of by gradient. The sources of all other chemicals and enzymes have been given (Van den Berghe *et al.*, 1980).

Experiments with isolated hepatocytes

White Leghorn chickens, weighing approx. 1.5 kg, were used after a 48 h fast. Hepatocytes were prepared as given previously (Van den Berghe *et al.*, 1980), except for the modifications such as retrograde perfusion required by the absence of a diaphragm from the avian body (Mapes & Krebs, 1978). Samples of up to 10 ml, corresponding to 0.5–1 g of cells, were incubated in Krebs–Henseleit (1932) bicarbonate buffer containing

15 mM-glucose. Radioactive precursors and enzyme inhibitors were added as indicated in the legends to the figures. Except for the experiments depicted in Fig. 1 and Table 1, this was followed by 15 min of preincubation and subsequent washing of the cells in buffer. The methods used for the extraction of metabolites have been given in detail (Van den Berghe *et al.*, 1980). Uric acid was determined by the method of Liddle *et al.* (1959). ATP, ADP and AMP were measured by enzymic methods and, in some experiments, by h.p.l.c. as given previously (Van den Berghe *et al.*, 1980). The radioactivity in the adenine nucleotides was determined after their one-dimensional separation on polyethyleneimine–cellulose thin-layer plates, and that in adenine, adenosine, the sum of inosine, hypoxanthine and xanthine, and in uric acid, after chromatography on cellulose thin-layer plates (Crabtree & Henderson, 1971). The concentrations of radioactive nucleotides, nucleosides and bases over the course of the experiments were calculated from the specific radioactivity of ATP determined at zero time. Unless given otherwise, the results shown are representative of two to three experiments.

Measurement of enzyme activities

Adenosine deaminase and AMP deaminase were assayed at a substrate concentration of 0.2 mM in 50 mM-imidazole buffer (pH 6.5)/0.1 M-KCl, with an Aminco DW-2 dual-wavelength spectrophotometer by the methods described by Schultz & Lowenstein (1976). Coformycin, when added, was preincubated for 15 min with the enzyme preparations, before starting the reaction with substrate.

RESULTS

Some characteristics of purine metabolism in isolated chicken hepatocytes

At the beginning of the incubations, the concentrations of the adenine nucleotides in the cells, expressed as $\mu\text{mol/g}$ wet wt., were 2.14 ± 0.18 for ATP, 1.07 ± 0.17 for ADP and 0.27 ± 0.08 for AMP (means $\pm$ S.E.M. for five or six preparations). These values are comparable with those reported by others in the same preparation (Dickson & Langslow, 1978; Burns & Buttery, 1981). They are also similar to those measured in freeze-clamped liver taken from artificially ventilated chicken (Tinker *et al.*, 1984), indicating that, notwithstanding the temporary anoxia accompanying their preparation, isolated chicken hepatocytes can be considered appropriate to investigate adenine nucleotide metabolism in uricotelic species. The nucleotide concentrations were not significantly modified over the 60 min duration of the experiments (see, e.g., Fig. 3a). Urate was produced at a rate which tended to accelerate at the end of the incubation and reached 37.1 ± 4.0 nmol/min per g wet wt. of cells (mean $\pm$ S.E.M., $n = 7$) over 60 min.

Labelled adenine and adenosine, at 1 μM concentrations, and hypoxanthine, at 5 μM concentration, added to the cell suspension, were rapidly metabolized (Fig. 1). Disappearance rates of the three precursors, calculated over the first 2 min and expressed as nmol/min per g of cells, were 4.2, 5.7 and 20 respectively. Within 5–10 min, 70–80% of 1 μM -[^{14}C]adenine (Fig. 1a) and of 1 μM -[^{14}C]adenosine (Fig. 1b) were incorporated in the cellular nucleotides, a process known to occur by way of adenine

Fig. 1. Time course of the conversion of adenine (a), adenosine (b) and hypoxanthine (c) into adenine nucleotides and uric acid in isolated chicken hepatocytes

[^{14}C]Adenine (465 c.p.m./pmol) and [^{14}C]adenosine (750 c.p.m./pmol) were added at a final concentration of $1\ \mu\text{M}$, and [^{14}C]hypoxanthine (80 c.p.m./pmol) at $5\ \mu\text{M}$.

phosphoribosyltransferase (not shown in Scheme 1) and adenosine kinase respectively. Later, the radioactivity in the adenine nucleotides decreased slowly, by approx. 5% over the duration of the experiment. With both precursors, radioactivity in uric acid accumulated in a biphasic way: a faster initial rate, recorded as long as precursor was still present in the suspension, was followed by a slower rate. The fast initial rate of accumulation probably reflects a more direct conversion of the highly radioactive precursor into uric acid. Calculated from the specific radioactivity of the precursor, this conversion would not exceed about 0.12 nmol/min per g of cells with adenine and 0.20 nmol/min per g of cells with adenosine. With adenosine it most likely results from the successive action of adenosine deaminase, nucleoside phosphorylase and xanthine oxidase (Scheme 1); with adenine it indicates that, contrary to common belief (Zielke & Suelter, 1971), traces of adenine deaminase (not shown in Scheme 1) may be present in certain animal tissues. Once the precursor had disappeared from the suspension, the rate of accumulation of radioactivity in uric acid approximately mirrored the decrease in radioactivity in the adenine nucleotides. As calculated from the specific radioactivity of the latter, which was about 300-fold lower than that of the precursor, the production of labelled uric acid then reached approx. 5 nmol/min per g of cells. This compares with a rate of production of allantoin of around 12 nmol/min per g of cells by isolated rat hepatocytes (Bontemps *et al.*, 1983). It can thus be deduced that the catabolism of the adenylate pool contributes slightly less than 15% to the total production of uric acid by isolated chicken hepatocytes and that the rate of turnover of their adenylates is much slower than the rate of the 'de novo' synthetic pathway. The latter conclusion was confirmed by the finding that, after

Fig. 2. Influence of coformycin on the activities of chicken liver adenosine deaminase and AMP deaminase

The activities of both enzymes were measured as given in the Materials and methods section. The 100% values, expressed as nmol/min per mg of protein, were 2.9 for adenosine deaminase (●) and 5.2 for AMP deaminase (○) in the crude extract, and 1900 for the partially purified AMP deaminase (△).

labelling with $1\ \mu\text{M}$ -[^{14}C]adenine (Fig. 1a), the specific radioactivity of the adenine nucleotides decreased by $7.7 \pm 1.3\%$ (mean $\pm$ S.E.M., $n = 6$) from 5 to 60 min of incubation, whereas during the same time interval that of uric acid decreased approx. 2.5-fold.

In contrast with adenine and adenosine, less than 5% of $5\ \mu\text{M}$ -[^{14}C]hypoxanthine was incorporated in the cellular adenine nucleotides, and the purine base was rapidly completely oxidized to uric acid (Fig. 1c). These

Fig. 3. Effects of coformycin and of 5-iodotubercidin on adenine nucleotide catabolism

The hepatocytes were preincubated for 15 min with $1 \mu\text{M}$ - ^{14}C adenine, in the absence (a, c, e, g) or in the presence of $1 \mu\text{M}$ -coformycin (b, d, f, h), followed by washing with inhibitor-free medium. Iodotubercidin ($100 \mu\text{M}$; c, d, g, h) was added at zero time. Panels (a)–(d) depict the total concentration of the metabolites; panels (e)–(h) represent the concentrations of the radioactive compounds, calculated from the specific radioactivity of ATP at zero time. The suspension contained approx. 85 mg of cells/ml. Key: ●, ATP + ADP + AMP; ○, ATP; ▲, uric acid; △, adenosine.

results are comparable with those obtained with isolated rat hepatocytes, indicating that chicken liver hypoxanthine–guanine phosphoribosyltransferase, similarly to the rat enzyme, is profoundly inhibited under physiological conditions (Vincent *et al.*, 1984).

Determination of the concentrations of coformycin and of 5-iodotubercidin required to inhibit chicken liver enzymes

In order to determine the concentration of coformycin required to inhibit either adenosine deaminase alone, or both the latter enzyme and AMP deaminase (Van den Berghe *et al.*, 1980), the influence of increasing concentrations of the inhibitor was investigated on the activity of the two enzymes in a crude chicken liver extract. A partially purified preparation of AMP deaminase was also used. As shown in Fig. 2, chicken liver adenosine deaminase required $1 \mu\text{M}$ -coformycin for maximal inhibition, which is 10-fold the concentration needed for maximal inhibition of the rat liver enzyme (Van den Berghe *et al.*, 1980). At $1 \mu\text{M}$, coformycin inhibited the activity of AMP deaminase in the crude extract by approx. 55% and that of partially purified AMP deaminase by 40%. The more pronounced inhibition recorded in the crude extract is most likely explained by an interference by the more coformycin-sensitive adenosine deaminase in the spectrophotometric assay, owing

to parallel dephosphorylation of AMP to adenosine. At $100 \mu\text{M}$ -coformycin, both the crude and the purified AMP deaminase preparations were inhibited by more than 90%.

The inhibitory effect of 5-iodotubercidin on adenosine kinase was assessed by its capacity to inhibit the incorporation of ^{14}C adenosine into the adenine nucleotides of the isolated hepatocytes. At $100 \mu\text{M}$, 5-iodotubercidin decreased the rate of incorporation of $1 \mu\text{M}$ - ^{14}C adenosine into the adenine nucleotides by 92%, from 5 to 0.4 nmol/min per g of cells (results not shown).

Influence of coformycin and of 5-iodotubercidin on adenine nucleotide catabolism in isolated chicken hepatocytes

Fig. 3 depicts the influence of both inhibitors alone and in association on the concentration of adenine nucleotides in the hepatocytes and on that of uric acid and adenosine in the cell suspension. Panels (a)–(d) show the time course of changes in total concentrations, whereas panels (e)–(h) depict the concentrations of the radioactively labelled compounds. Coformycin ($1 \mu\text{M}$) modified neither the total concentration of the adenine nucleotides nor the total rate of production of uric acid (Fig. 3b). In the presence of coformycin (Fig. 3f), as in its absence (Fig. 3e), radioactive adenosine was undetect-

Fig. 4. Influence of anoxia on adenine nucleotide catabolism

The hepatocytes were preincubated for 15 min in O_2/CO_2 with $1 \mu M$ - $[^{14}C]$ adenine, in the absence (a, d) or in the presence of coformycin at $1 \mu M$ (b, e) or $100 \mu M$ (c, f), followed by washing with inhibitor-free medium. At zero time, the gas phase was replaced by N_2/CO_2 . Panels (a)–(c) depict total concentrations ($\circ$, ATP; $\square$, ADP; $\triangle$, AMP; $\bullet$, ATP+ADP+AMP) and panels (d)–(f) those of the radioactive compounds ($\blacktriangle$, uric acid; $\bullet$, inosine+hypoxanthine+xanthine; $\triangle$, adenosine), calculated from the specific radioactivity of ATP at zero time. The suspension contained approx. 75 mg of cells/ml.

able. The inhibitor also did not influence the concentration of the labelled adenine nucleotides and the production of radioactive uric acid (Fig. 3f). The latter finding indicates that in isolated chicken hepatocytes, as in rat liver cells (Van den Berghe *et al.*, 1980), the formation of the terminal purine catabolite does not proceed via adenosine deaminase under physiological conditions. Since this observation implies that the catabolism of AMP proceeds by way of AMP deaminase, the observation that the production of radioactive uric acid was not influenced by $1 \mu M$ -coformycin allows one to conclude that the activity of AMP deaminase inside the cells, unlike that in the crude extract, was not influenced by that concentration of the inhibitor.

Addition of $100 \mu M$ -5-iodotubercidin provoked a 3-fold increase in the formation of labelled uric acid, an accumulation of labelled adenosine up to 9 nmol/ml of cell suspension over 60 min, and a 45% decrease in the concentration of radioactive adenylates over the same time interval (Fig. 3g). All these results can be explained by the known inhibitory effect of 5-iodotubercidin on

adenosine kinase (Henderson *et al.*, 1972). They indicate that adenosine is normally formed in the cells, but does not contribute to the formation of uric acid, because it is rephosphorylated by adenosine kinase (step 8 in Scheme 1). Unexpectedly, and in contrast with isolated rat hepatocytes (Bontemps *et al.*, 1983), 5-iodotubercidin barely influenced the total concentration of ATP, the specific radioactivity of which decreased by 33% over the duration of the experiment. It also markedly diminished the total uric acid production (Fig. 3c). This indicates that in the presence of 5-iodotubercidin there was also an increase in the conversion of IMP, provided by the 'de novo' pathway, into adenylates, and a diminution of its hydrolysis to inosine. The observation that the decrease in the total production of uric acid was more pronounced than the formation of unlabelled adenylates indicates that, in addition, 5-iodotubercidin could inhibit the pathway leading directly from 5-phosphoribosyl 1-pyrophosphate to inosine (steps 1 and 6 in Scheme 1). Since 5-iodotubercidin had no effect on the activity of the cytoplasmic 5'-nucleotidase from rat liver (Bontemps

Table 1. Influence of anoxia and reoxygenation on the concentration and on the specific radioactivity of adenine compounds in isolated chicken hepatocytes in the absence and in the presence of 1 μ M- or 100 μ M-coformycin

The cells were incubated for 15 min in O₂/CO₂ in the presence of 1 μ M-[¹⁴C]adenine and of the concentrations of coformycin indicated, before the measurements in normoxia. Thereafter anoxia was provoked by replacement of the O₂/CO₂ gas phase by N₂/CO₂. After 60 min, reoxygenation was accomplished by re-introducing the O₂/CO₂ gas phase for 30 min. Concentrations are expressed as μ mol/g wet wt. of cells. For the adenylates total concentrations are given, whereas for adenosine the concentration of the radioactive compound was calculated from the specific radioactivity of ATP after 15 min of normoxia: n.d., not detectable.

	Coformycin (μ M)	After 15 min of normoxia	After 60 min of anoxia	After 30 min of reoxygenation
ATP (μ mol/g)	0	2.06	0.19	1.56
	1	1.95	0.21	1.76
	100	1.91	0.09	2.24
ADP (μ mol/g)	0	1.30	0.26	0.56
	1	1.55	0.34	0.53
	100	1.46	0.37	0.79
AMP (μ mol/g)	0	0.20	0.46	0.12
	1	0.27	0.46	0.18
	100	0.30	0.65	0.39
Total adenylates (μ mol/g)	0	3.56	0.91	2.24
	1	3.77	1.01	2.47
	100	3.67	1.11	3.42
Sp. radioactivity of ATP (c.p.m./nmol)	0	1735	1118	225
	1	1705	1223	203
	100	1764	1534	1299
Adenosine (μ mol/g)	0	n.d.	0.07	0.03
	1	n.d.	0.67	0.06
	100	n.d.	2.31	0.59

et al., 1983), we suspect that it inhibits one of the enzymes of the 'de novo' pathway.

When 5-iodotubercidin and coformycin were added together, the changes in concentrations of total and radioactive ATP (Figs. 3d and 3h) and in the specific radioactivities of the adenine nucleotides and of uric acid were similar to those recorded in the presence of 5-iodotubercidin alone. However, the production of total uric acid was decreased 2-fold and that of radioactive uric acid was diminished 3-fold as compared with 5-iodotubercidin alone. This can be explained by the impairment, by 1 μ M-coformycin, of the degradation of adenosine, accumulating because of the addition of 5-iodotubercidin. Indeed, radioactive adenosine now increased to 23 nmol/ml at 60 min (Fig. 3h).

Influence of anoxia on adenine nucleotide catabolism in isolated chicken hepatocytes

As shown in the representative experiment depicted in Fig. 4, replacement of the O₂/CO₂ gas phase by N₂/CO₂ in the incubation medium of the liver cells resulted in an approx. 4-fold decrease in the concentrations of ATP and ADP, accompanied by a 6-fold increase in AMP over 10 min. Thereafter the concentration of AMP decreased progressively, reaching nearly its normal value at 60 min. Total adenylates decreased about 3-fold over the whole duration of the experiment (Fig. 4a). Remarkably, total uric acid production was not modified significantly as compared with incubation in O₂/CO₂, but radioactive uric acid now accounted for 85% of this production, instead of 14% in normoxic conditions. This indicates that adenine nucleotide catabolism rather than synthesis

de novo now provided most of the uric acid produced by the cells. There was also an accumulation of labelled adenosine, to 25 nmol/ml of cell suspension at 20 min, which decreased progressively thereafter (Fig. 4d). This shows that the degradation of AMP induced by anoxia also proceeds by way of the cytoplasmic 5'-nucleotidase acting on AMP. Radioactive inosine, hypoxanthine and xanthine together reached a concentration close to that of uric acid.

In the presence of 1 μ M-coformycin, the decreases in ATP and ADP and the accumulation of AMP induced by anoxia were not significantly modified (Fig. 4b). However, labelled adenosine now accumulated to a concentration of 50 nmol/ml of cell suspension at 20 min, which was maintained thereafter (Fig. 4e). In contrast, the accumulation of inosine, hypoxanthine and xanthine together was decreased by 50%. Both the total production of uric acid (Fig. 4b) and that of radioactive uric acid (Fig. 4e) were slightly decreased. All these results confirm the conclusion that the initial degradation of AMP induced by anoxia proceeds by way of both the cytoplasmic 5'-nucleotidase and AMP deaminase.

On addition of 100 μ M-coformycin, the concentration of AMP, after its initial elevation, decreased slightly more slowly (Fig. 4c), whereas labelled adenosine became the major end product of nucleotide catabolism and accumulated to a concentration of 175 nmol/ml of cell suspension at 60 min (Fig. 4f). The concentrations of labelled inosine, hypoxanthine and xanthine together, as well as of radioactive uric acid, were decreased to 10–15% of those recorded in the absence of coformycin. These results show that in the presence of 100 μ M-

coformycin both the deamination of adenosine and that of AMP were profoundly inhibited. The observation that the total production of uric acid remained higher than that of the radioactive compound in anoxia indicates that synthesis of purines *de novo* still proceeded under this condition. This is confirmed by the finding that anoxia induced an approx. 30% decrease in the specific radioactivity of the adenine nucleotides over 60 min (see also Table 1). The decrease in specific radioactivity of the adenine nucleotides was not modified by the addition of 1 μ M-coformycin, but was about halved in the presence of 100 μ M inhibitor, in accordance with a preservation of the adenine nucleotide pool produced by inhibition of AMP deaminase.

Table 1 also gives the results of a single experiment in which incubation of the cells in N_2/CO_2 for 60 min was followed by 30 min of reoxygenation in O_2/CO_2 . In the absence of coformycin, reoxygenation resulted in an increase of the concentration of ATP to 75% and of that of ADP to 40% of its control value, whereas the concentration of AMP decreased nearly 4-fold. About 60% of the total adenine nucleotide pool, of which 75% had been lost, was regenerated at the end of the experiment. Addition of 100 μ M-coformycin induced nearly full recovery of the concentrations of the adenine nucleotides after reoxygenation. In the absence of coformycin, reoxygenation was accompanied by a marked 5-fold decrease in the specific radioactivity of ATP. This decrease was less pronounced in the presence of 1 μ M- and particularly of 100 μ M-coformycin. These results show that, in the absence of coformycin, reoxygenation induced a replenishment of the adenine nucleotide pool by markedly stimulating the '*de novo*' synthetic pathway, whereas in the presence of the inhibitor accumulated adenosine also provided a significant contribution.

DISCUSSION

In contrast with lipogenesis (Goodridge, 1973; Capuzzi *et al.*, 1974), gluconeogenesis (Dickson & Langslow, 1978; Mapes & Krebs, 1978) and the production of urate from amino acids (Mapes & Krebs, 1978; Burns & Buttery, 1981), the metabolism of the adenine nucleotides has been little investigated in isolated chicken hepatocytes. In this study we have obtained information concerning: (1) the turnover of the adenine nucleotide pool as compared with the rate of synthesis *de novo*; (2) the pathway of the initial degradation of AMP in the presence and in the absence of oxygen; (3) the control of the conversion of IMP, provided by the '*de novo*' pathway, into adenylates. These three points will be discussed separately.

(1) Turnover of the adenine nucleotides under physiological conditions

Our results show that isolated chicken hepatocytes maintain a remarkably stable adenine nucleotide pool during a 60 min incubation in an O_2/CO_2 gas phase (Figs. 3a and 3e). The rate at which they produce uric acid, namely 37 nmol/min per g of cells, thus closely reflects the velocity of the '*de novo*' synthesis of IMP. The small, approx. 8%, decrease of the specific radioactivity of the adenine nucleotides over the 55 min after the incorporation of 1 μ M- $[^{14}C]$ adenine allows us to calculate that the adenine nucleotide pool, which

amounts to about 3.5 μ mol/g of cells, is renewed at a rate of approx. 5 nmol/min per g of cells. It can thus be concluded that, of the IMP synthesized by the '*de novo*' pathway, about 14% is channelled into the adenine nucleotide pool, the remainder being hydrolysed directly to inosine. This indicates the existence of mechanisms that control the conversion of IMP, either into AMP and the other adenylates, or into inosine and the further products of purine catabolism. These controls should be located at the level of adenylosuccinate synthetase and of the cytoplasmic 5'-nucleotidase, which are both highly regulated enzymes, in rat as well as in chicken liver (Stayton *et al.*, 1983; Van den Berghe *et al.*, 1977b; Itoh *et al.*, 1978). The 7-fold slower turnover of the adenine nucleotide pool of isolated chicken hepatocytes as compared with the rate of their purine synthesis *de novo* also implies a limitation of the degradation of the adenylates.

(2) AMP-degradative pathways

The observation that, under physiological conditions, 1 μ M-coformycin did not modify the production of total as well as of labelled uric acid (Figs. 3b and 3f) indicates that the degradation of AMP, leading to the production of uric acid by isolated chicken hepatocytes, does not proceed by way of the sequence 5'-nucleotidase/adenosine deaminase. The 40% decrease in $[^{14}C]$ ATP, accompanied by a 3-fold increase in the production of labelled uric acid, provoked by the adenosine kinase inhibitor 5-iodotubercidin (Fig. 3g), indicates nevertheless that adenosine is normally formed in the cells by the cytoplasmic 5'-nucleotidase, but does not contribute to the formation of uric acid because it is rephosphorylated by adenosine kinase. This substrate cycle, which was demonstrated in isolated rat hepatocytes (Bontemps *et al.*, 1983), thus also operates in chicken liver cells. From the increase in the production of radioactive uric acid induced by 5-iodotubercidin it can be calculated that the activity of this futile cycle reaches at least 11 nmol/min per g of cells, i.e. twice the rate of adenylate catabolism.

In contrast with normoxic hepatocytes, the production of radioactive uric acid by anoxic cells was decreased by 20% when adenosine deaminase was inhibited by 1 μ M-coformycin (Fig. 4e). This indicates that the degradation of AMP induced by anoxia in chicken liver cells also proceeds by way of the 5'-nucleotidase acting on AMP. This is in contrast with rat hepatocytes subjected to anoxia, in which the production of uric acid was not influenced by inhibition of adenosine deaminase (Vincent *et al.*, 1982). The difference may be explained by a more active 5'-nucleotidase (Naito *et al.*, 1974) or by a less active recycling of adenosine in chicken hepatocytes as compared with rat liver cells. The observation that adenosine accumulated in the absence (Figs. 4d) and in the presence of 1 μ M-coformycin (Fig. 4e) in anoxic chicken hepatocytes, but only at 100 μ M-coformycin in oxygen-deprived rat liver cells (Vincent *et al.*, 1982) accords with this interpretation.

As opposed to anoxic hepatocytes from fasted rats, in which the elevated concentration of AMP remained virtually unchanged over 50 min (Vincent *et al.*, 1982), AMP in anoxic chicken hepatocytes decreased to nearly control values over the same time interval (Figs. 4a-4c). The more rapid rate of disappearance of AMP in the latter cells may be explained by the existence of two forms of AMP deaminase in chicken liver (Spychala &

Makarewicz, 1983). Form I displays allosteric properties that are qualitatively similar to those of rat liver AMP deaminase, namely a sigmoid saturation curve for AMP in the absence of effectors, a stimulation by ATP and an inhibition by GTP and P_i (Van den Berghe *et al.*, 1977a). These properties result in a profound inhibition of the enzymic activity under physiological conditions and in an even more marked suppression thereof under anoxic conditions, because of the decrease in ATP and concomitant increase in P_i . In contrast, form II of chicken liver AMP deaminase displays hyperbolic kinetics and a lower K_m for AMP, is less ATP-dependent, and may be less effective in controlling the degradation of AMP when its concentration is elevated. Another factor in the more rapid catabolism of AMP in isolated chicken hepatocytes may be the above-mentioned several-fold higher activity of the cytosolic 5'-nucleotidase in chicken as compared with rat liver (Naito *et al.*, 1974). The accumulation of inosine, hypoxanthine and xanthine (Figs. 4d and 4e) in anoxic chicken hepatocyte suspensions, a finding that was not recorded in rat preparations (Vincent *et al.*, 1982), shows that in chicken liver cells xanthine oxidase becomes limiting under conditions of accelerated nucleotide breakdown.

(3) Control of the rate of conversion of IMP into AMP

The observation that in isolated hepatocytes incubated in oxygen the total concentration of ATP was barely modified in the presence of 5-iodotubercidin (Fig. 3c), but that its specific radioactivity decreased (Fig. 3g), indicates that the loss of ATP owing to inhibition of adenosine kinase was almost exactly compensated by synthesis *de novo* of unlabelled adenylates. Since in the presence of 5-iodotubercidin the production of radioactive uric acid accounted for 65% of the total production of the purine catabolite (cf. Figs. 3c and 3g), as opposed to 14% in its absence, it can be concluded that the inhibitor provoked the conversion of 4–5-fold more of the IMP synthesized by the '*de novo*' pathway into adenylates. This increased conversion of IMP into AMP was accompanied by a decrease in the production of total uric acid, which does not seem to be caused by an impairment of the conversion of IMP into inosine, but by an inhibition of the '*de novo*' pathway. We have no explanation as yet for the decrease of the rate of synthesis of IMP *de novo* and for the enhancement of its rate of conversion into AMP, observed in the presence of 5-iodotubercidin. However, the latter observation shows that the channelling of IMP into adenylates can be increased when the adenine nucleotide pool is jeopardized. The results of studies in which the concentration of oxygen in the gas phase was modified confirm this conclusion. The 30% diminution of the specific radioactivity of the adenine nucleotides recorded after 60 min of anoxia in the absence and in the presence of 1 μ M-coformycin (Table 1), as compared with about 8% under control conditions, indicates an increase in the conversion of IMP, provided by the '*de novo*' pathway, into adenine nucleotides. This flux is further enhanced during reoxygenation, as evidenced by the marked additional decrease of the specific radioactivity of ATP under the latter condition (Table 1). From the increase in the sum of the adenine nucleotides recorded over

30 min of reoxygenation, it can be calculated that the rate of conversion of IMP into AMP then reaches 42 nmol/min per g of cells, an 8-fold higher rate than in normoxic control conditions. This modification implies an enhancement of the activity of adenylosuccinate synthetase, which may be caused by various mechanisms: (1) an increase in free Mg^{2+} , a known stimulator of the enzyme (Stayton *et al.*, 1983), resulting from the degradation of the Mg^{2+} chelator ATP; (2) an increase in the concentration of its substrate, IMP; (3) particularly during reoxygenation, the decrease in AMP, one of the inhibitors of the enzyme.

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