The Effects of Added Purines on Urate and Purine Synthesis *de novo* by Isolated Chick Liver, Kidney and Lymphoid Cells

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1. Isolated chick lymphoid cells, together with isolated chick liver and kidney cells, incorporate $[1-^{14}\text{C}]$ glycine or $[^{14}\text{C}]$ formate into urate. 2. Of the cell types used, bursal cells incorporate ^{14}C into urate at the fastest rate, although the output of total urate by bursal cells is only 10% that of liver cells. 3. When suspended in Eagle's medium the incorporation of ^{14}C into urate is inhibited by adenine and guanine up to 1 mM. In contrast, the addition of 1 mM-AMP or -GMP results in a relatively large stimulation of this incorporation. 4. Added adenine is rapidly taken up by liver cells and then released in an unmetabolized form; AMP is taken up more slowly and is rapidly metabolized. The metabolites (possibly including adenine) are then released. 5. Intracellular liver 5-phosphoribosyl 1-pyrophosphate is approx. 0.7 mM and remains constant or falls slightly during a 3 h incubation of the cells. 6. The addition of adenine or guanine, AMP or GMP, does not alter liver intracellular 5-phosphoribosyl 1-pyrophosphate concentrations. Added 5-phosphoribosyl 1-pyrophosphate is not taken up by liver cells. 7. The results are discussed in the context of the control of urate and purine synthesis *de novo* in the chick.

We have shown that isolated chick liver and kidney cells incorporate $[1-^{14}\text{C}]$ glycine into urate, and that this was inhibited by 1 mM concentrations of AMP and of GMP, in liver but not in kidney cells (Badenoch-Jones & Buttery, 1975a). These experiments were carried out with the cells suspended in a simple balanced salt solution, Hepes*-buffered Hanks' medium. We have re-investigated this effect by using a more complex, more 'physiological' medium, namely Eagle's (1955) minimal essential medium supplemented with glycine.

Work on Ehrlich ascites-tumour cells (Henderson *et al.*, 1975; Bagnara *et al.*, 1974) has suggested that the intracellular concentration of 5-phosphoribosyl 1-pyrophosphate is of greater importance than feedback inhibition in controlling the rate of purine synthesis *de novo* in intact cells. The pathways of urate and purine-base synthesis *de novo* in the chick are thought to be identical through to inosinic acid (Hartman, 1970). Hence, as urate is the main nitrogenous excretory product in the chick, it might be expected that neither feedback inhibition nor the availability of 5-phosphoribosyl 1-pyrophosphate would be rate-limiting for the synthesis *de novo*. In this context we have measured intracellular liver

5-phosphoribosyl 1-pyrophosphate concentrations to see if variations in these might account for the effects of added purines on urate and purine synthesis *de novo* that we have observed.

In addition, there is a lack of information on the capacity of extrahepatic tissues to synthesize urate *de novo*, although we have shown that isolated kidney cells incorporate $[1-^{14}\text{C}]$ glycine into urate (Badenoch-Jones & Buttery, 1975a). We have now investigated the ability of lymphoid cells to do this.

Materials and Methods

Materials

Eagle's (1955) minimum essential medium buffered with Hepes was obtained from Flow Laboratories Ltd., Irvine, Scotland, U.K. This was supplemented with 1 mM-glycine before use, as glycine is an essential amino acid in the chick (Scott *et al.*, 1969), but not in mammalian cells, for which Eagle's medium was designed. Ficoll (mol. wt. 70000) was from Pharmacia, Uppsala, Sweden.

$[8-^{14}\text{C}]$ Adenine (51 $\mu\text{Ci}/\mu\text{mol}$), $[2-^{14}\text{C}]$ uric acid (50 $\mu\text{Ci}/\mu\text{mol}$), $[^{14}\text{C}]$ formic acid (sodium salt; 58 $\mu\text{Ci}/\mu\text{mol}$), $[1-^{14}\text{C}]$ glycine (53.8 $\mu\text{Ci}/\mu\text{mol}$) and $[\text{U}-^{14}\text{C}]$ -AMP (450 $\mu\text{Ci}/\mu\text{mol}$) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The cation-exchange resin AG 50W (X8, H^+ form,

* Abbreviation: Hepes, 2-(N-2-hydroxyethyl)piperazin-N'-yl)ethanesulphonic acid.

400 mesh) was supplied by Bio-Rad Laboratories, Richmond, CA, U.S.A. The 5-phosphoribosyl 1-pyrophosphate standard was obtained from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K. The purity, as assayed by Sigma, was 80%, and this value was used for all calculations. Other materials used were as previously described (Badenoch-Jones & Buttery, 1975a,b).

Cell preparations

Liver and kidney cells were prepared and counted, and Trypan Blue exclusion was measured as previously described (Badenoch-Jones & Buttery, 1975a,b). Lymphoid cells were prepared by mechanical disruption of the tissues. Spleen, thymus and bursa were removed, placed in Eagle's minimum essential medium and cut up with scissors. The tissue pieces were gently compressed between a plastic syringe barrel and the bottom of a Petri dish. The suspension was filtered through gauze, centrifuged at $500g_{\max}$ for 5 min and the pellet resuspended at a concentration of 15×10^6 – 30×10^6 cells/ml in Eagle's minimum essential medium. Viability, as judged by Trypan Blue exclusion, was better than 95%. Cell volumes were measured by using a micrometer eyepiece; cell diameter was measured, and assuming the cells to be spheres, cell volume was calculated.

Purine and urate synthesis de novo

The incorporation of [$1-^{14}\text{C}$]glycine into adenine and guanine was measured by a method based on that of Smith & Markham (1950) for separating adenine and guanine from the cell suspension. To 1 ml of cell suspension was added 2 ml of 6% (w/v) HClO_4 , the mixture was then heated at 90°C for 30 min to hydrolyse nucleic acid, nucleosides and nucleotides to the free purine bases. The cooled suspension was centrifuged to remove the precipitated protein and the supernatant adsorbed on a column (5 mm $\times$ 30 mm) of Dowex AG 50W (X8) cation-exchange resin previously washed with 10 ml of 1 M-HCl. The resin was washed with 8 ml of 1 M-HCl, the adsorbed purine bases then being eluted with 6 ml of 6 M-HCl.

The 6 M-HCl eluates were evaporated to dryness in watch glasses on a boiling-water bath; the solid material was then dissolved in 100 μl of 1 M-HCl. A portion (20 μl) was applied to Whatman no. 1 paper and separated by ascending chromatography in propan-2-ol/water (7:3, v/v). The chromatographic solvent used was suitable for our requirements to separate adenine and guanine from glycine, urate, formate and possibly serine (produced from glycine). In the system used R_F values were: adenine, 0.50; guanine, 0.50; glycine, 0.22; urate, 0.18; formate, 0.36; serine, 0.21. The samples were run overnight and the chromatograms scanned for radioactivity with a Nuclear-Chicago Actigraph scanner. The total radioactivity in the combined adenine and guanine

peak was quantified by using a disc-integrator (Disc Instrument Inc., Santa Ana, CA, U.S.A.) compared with a known ^{14}C standard.

By this method the recovery of [^{14}C]adenine and [^{14}C]guanine was $84 \pm 8\%$ (4).

The incorporation of [$1-^{14}\text{C}$]glycine or [^{14}C]formate into urate was measured by precipitation of mercuric urate, as previously described (Badenoch-Jones & Buttery, 1975a). As it was important to ensure a clean separation of urate from the purine bases, control experiments were carried out with [$2-^{14}\text{C}$]uric acid and [$8-^{14}\text{C}$]adenine. These showed that although the recovery of [$2-^{14}\text{C}$]urate as the mercuric precipitate was $89 \pm 5\%$ (7) between 1 pmol and 1 nmol, some [$8-^{14}\text{C}$]adenine was also precipitated. In the absence of uric acid, approx. 5% of adenine was precipitated from 1 ml of a solution of up to 0.5 mM; in the presence of uric acid this percentage was increased. Hence, although the method gives a quantitative recovery of urate, there is a variable recovery of adenine also. In view of this, the cell extracts were first hydrolysed with HClO_4 and adenine and guanine removed by adsorption on to the Dowex column as above. The 1 M-HCl washings were then used for mercuric precipitation of the urate. Retention of [$2-^{14}\text{C}$]urate by the column was not measurable. Results for the ^{14}C content of the mercuric precipitate of the untreated cell extract were about 10% higher than in cell extracts first adsorbed on to the Dowex column.

5-Phosphoribosyl 1-pyrophosphate assay

5-Phosphoribosyl 1-pyrophosphate was assayed by the method of Bagnara *et al.* (1974) modified during the present study for use with chick liver cells. This involves measuring the 5-phosphoribosyl 1-pyrophosphate-dependent conversion of [$8-^{14}\text{C}$]adenine into [^{14}C]AMP. A portion (200 μl) of cell suspension was heated in a boiling-water bath for 20 s for optimum extraction of 5-phosphoribosyl 1-pyrophosphate; to this was added 10 μl of an aqueous solution of [$8-^{14}\text{C}$]adenine (1 mM; 10 $\mu\text{Ci/ml}$) and 40 μl of liver extract. The extract was prepared as follows. A chick liver was removed, perfused with ice-cold Eagle's minimum essential medium until blanched and then dispersed by using a glass/Teflon Potter-Elvehjem homogenizer, as a 1:3 (w/v) homogenate. This was then centrifuged at $1000g_{\max}$ for 5 min and the supernatant stored at -20°C until required.

The mixture was incubated at 40°C for 60 min and the reaction stopped by the addition of 25 μl of 8 M-formic acid. A 50 μl sample was separated on Whatman no. 1 paper by descending chromatography in propan-1-ol/30% (w/w) NH_3 /water (6:3:1, by vol.). The chromatograms were scanned by using a Nuclear-Chicago Actigraph scanner. R_F values in this system were: adenine, 0.56; AMP, 0.34; ADP, 0.30;

ATP, 0.28; IMP, 0.28. The combined radioactive peaks of material corresponding to AMP, ADP, ATP and IMP was quantified by using a Disc-integrator and compared with results obtained by using the 5-phosphoribosyl 1-pyrophosphate standard in the assay system.

The recovered nucleotide radioactivity increased linearly with 5-phosphoribosyl 1-pyrophosphate concentration over the range used, 0–5000 pmol per sample. The reproducibility of the assay was $\pm 17\%$ (5). When 5-phosphoribosyl 1-pyrophosphate was assayed in cell suspensions incubated with adenine, unused adenine was removed by the addition of 20 μ l of a 30% (v/v) suspension of activated charcoal (Norit A). 5-Phosphoribosyl 1-pyrophosphate was assayed in the clear supernatant after removal of the charcoal by centrifugation.

Separation of cells from the suspending medium

To measure intracellular 5-phosphoribosyl 1-pyrophosphate concentrations and uptake of adenine and AMP, the cells were separated from their suspending medium by rapid centrifugation through

Table 1. *Relative rates of incorporation of [1- 14 C]glycine into urate by different cell types*

Cells (10ml, at a concentration of 30×10^6 cells/ml) were incubated at 40°C with 2 μ Ci of [1- 14 C]glycine. The incorporation of [1- 14 C]glycine into urate was measured as described in the text. The rate of incorporation was linear with time for liver, kidney, spleen and thymus cells, but not for bursal cells (see Figs. 1 and 2), and are thus not strictly comparable. The quoted rates are the radioactivity (d.p.m.) incorporated after 3h divided by 3 to give the incorporation in d.p.m./h. Similar results were obtained by measuring the incorporation of [1- 14 C]formate into urate. Results are expressed as the mean $\pm$ S.E.M.

Cell type	Incorporation (d.p.m./h per 1×10^6 cells)	Protein (mg/ 10^6 cells)	Incorporation (d.p.m./h per mg of protein)
Liver	150 $\pm$ 42	0.50 $\pm$ 0.10	300
Kidney	100 $\pm$ 37	0.39 $\pm$ 0.11	260
Spleen	45 $\pm$ 19	0.10 $\pm$ 0.02	450
Thymus	47 $\pm$ 21	0.10 $\pm$ 0.02	450
Bursa	900 $\pm$ 202	0.10 $\pm$ 0.02	9000

a 5% (w/v) Ficoll solution (Badenoch-Jones & Buttery, 1975a).

Protein determination

Protein was determined by the method of Lowry *et al.* (1951), with dry bovine serum albumin as the standard.

Results

Urate synthesis by lymphoid cells

As shown in Fig. 1, bursa, spleen and thymus cells all incorporate [1- 14 C]glycine into urate. Table 1 shows the relative rates of incorporation for liver, kidney and lymphoid cells expressed per unit number of cells or per unit weight of protein. The rate of incorporation was linear with time for all the cells except bursa cells. These results show that bursa cells incorporate [1- 14 C]glycine into urate at a rate some 20–30-fold faster than do the other cells. This does not necessarily indicate a faster rate of actual synthesis of urate from glycine, but may be dependent on such factors as the rate of uptake of the glycine substrate and the size of the intracellular glycine pool.

The total output, however, of urate, as measured by using uricase (Kalckar, 1947) was 0.19 ± 0.03 (3) μ g/h per mg of protein, compared with a value of 2.0 ± 0.3 (11) μ g/h per mg of protein for liver cells.

Effect of AMP or GMP, adenine or guanine on incorporation of [1- 14 C]glycine and [1- 14 C]formate into urate

[1- 14 C]Glycine is incorporated into urate by liver cells at a constant rate under the conditions of

Fig. 1. *Relative incorporation of [1- 14 C]glycine into urate by bursa, spleen and thymus cells*

Cells (10ml, at a concentration of 15×10^6 /ml) were incubated with 2 μ Ci of [1- 14 C]glycine. Radioactive urate was precipitated as the mercuric salt as described in the text. The results were reproducible in three experiments, although the time at which the large increase in the rate of incorporation into bursa cells occurred varied from 30 to 150min. The proportion of Trypan Blue-staining cells increased from $4 \pm 1\%$ (3) to $16 \pm 3\%$ (3) during the 240min incubation for all three cell types. ●, Bursa; ■, thymus; ○, spleen.

Fig. 2. Effect of adenine and AMP on the incorporation of $[1-^{14}\text{C}]$ glycine into urate by liver cells

Cells (10ml, at a concentration of $30 \times 10^6/\text{ml}$) were incubated with $2\mu\text{Ci}$ of $[1-^{14}\text{C}]$ glycine in the absence (■) or presence of 1mM-AMP (○) or 1mM-adenine (●). $[^{14}\text{C}]$ Urate was isolated as the mercuric precipitate as described in the text. Results of one particular experiment are shown. In five experiments the inhibition with adenine was always linear with time, whereas the stimulation obtained with AMP was linear with time (two experiments) or increased with time (three experiments, as shown in this Figure). Percentage stimulation of inhibition of radioactivity incorporated into urate at 180min compared with the control was adenine $49 \pm 5\%$ (5) inhibition, AMP $105 \pm 21\%$ (5) stimulation. The additions did not alter the percentage of Trypan Blue-staining cells, which increased from $8 \pm 3\%$ (5) to $17 \pm 3\%$ (5) during 180min of incubation.

incubation used. As shown in Fig. 2, after a 3h incubation this incorporation is inhibited by $49 \pm 5\%$ (5) by 1mM-adenine and stimulated by $105 \pm 21\%$ (5) by 1mM-AMP. There is some variability in the kinetics of stimulation by AMP; this increased progressively with time (three experiments) or was constant with time (two experiments). A similar stimulation was obtained with 1mM-GMP as with AMP, and a similar inhibition was obtained with 1mM-guanine as with adenine (results not shown). The replacement of $[1-^{14}\text{C}]$ glycine by $[^{14}\text{C}]$ formate did not affect the results. This stimulation by AMP or GMP, and inhibition with adenine and guanine, was not specific to liver cells and was obtained with spleen and kidney cells also.

Effects of inosine, inosinic acid and 4-amino-5-imidazolecarboxamide ribonucleoside on incorporation of $[1-^{14}\text{C}]$ glycine into urate

Inosine, inosinic acid and 4-amino-5-imidazolecarboxamide ribonucleoside were added to a final concentration of 1mM to liver cells to determine whether compounds known to be rapidly converted into urate (Badenoch-Jones & Buttery, 1975b) affected the incorporation of $[1-^{14}\text{C}]$ glycine or $[^{14}\text{C}]$ formate into urate. The results are shown in Table 2; inosine and inosinic acid caused a large stimulation of incorporation, whereas 4-amino-5-imidazolecarboxamide ribonucleoside was inhibitory. None of the additions altered the percentage of Trypan Blue-staining cells, measured as previously described (Badenoch-Jones & Buttery, 1975b).

Effects of AMP and adenine on the incorporation of $[1-^{14}\text{C}]$ glycine into purine bases by liver cells

In view of the observed effects of AMP, GMP, adenine and guanine on the incorporation of $[1-^{14}\text{C}]$ glycine into urate, we have measured the incorporation of $[1-^{14}\text{C}]$ glycine into adenine and guanine to see if AMP or adenine affect this. AMP and adenine at 1mM had similar effects on the incorporation of glycine into adenine and guanine as on the incorporation into urate. The percentage stimulation in the presence of AMP was $92 \pm 12\%$ (3), and percentage inhibition in the presence of adenine was $41 \pm 13\%$ (3), compared with controls without added purine or nucleotide.

Uptake and metabolism of AMP and adenine by liver cells

To estimate the intracellular concentrations of adenine and AMP, which were altering $[1-^{14}\text{C}]$ glycine incorporation into both urate and adenine and guanine, their uptake into liver cells was measured. As shown in Fig. 3, adenine was rapidly taken up, the

Table 2. Effect of intermediates on the incorporation of $[1-^{14}\text{C}]$ glycine into urate by liver cells

Cells (10ml, at a concentration of $30 \times 10^6/\text{ml}$) were incubated at 40°C with $2\mu\text{Ci}$ of $[1-^{14}\text{C}]$ glycine in the presence or the absence of the additions shown. The incorporation of $[1-^{14}\text{C}]$ glycine into urate was determined as described in the text. Percentage change was calculated as $[(\text{test d.p.m.} - \text{control d.p.m.})/\text{control d.p.m.}] \times 100\%$ after 3h. Similar results were obtained by measuring the incorporation of $[^{14}\text{C}]$ formate into urate. Results are expressed as the mean $\pm$ S.E.M.

Addition (1 mM final concentration)	Change (%)
Inosine	$+86 \pm 13\%$ (3)
Inosinic acid	$+75 \pm 16\%$ (3)
4-Amino-5-imidazolecarboxamide ribonucleoside	$-50 \pm 10\%$ (3)

Fig. 3. Uptake of [8-¹⁴C]adenine (a) and [U-¹⁴C]AMP (b) by liver cells

Cells (10ml, at a concentration of $50 \times 10^6/\text{ml}$) were incubated with [8-¹⁴C]adenine (1 mM) or [U-¹⁴C]AMP (1 mM). Cells were separated from the suspension medium by rapid centrifugation through a 5% (w/v) Ficoll solution as described in the text. The cell pellet was suspended in 0.5 ml of 0.5M-formic acid and a sample counted for radioactivity. The results of three individual experiments are shown (●, ▲, ■) and expressed as the percentage of the total radioactivity taken up by the cell pellet with time. For comparison the uptake of [1-¹⁴C]glycine (1 mM) under the same conditions is shown (○).

Fig. 4. Metabolism of added adenine (●) and AMP (○) by liver cells

Cells (10ml, at a concentration of $50 \times 10^6/\text{ml}$) were incubated with [8-¹⁴C]adenine (1 mM) or [U-¹⁴C]AMP (1 mM). Samples (1 ml) were centrifuged rapidly through a 5% (w/v) Ficoll solution to separate the cell pellet, and 0.5M-formic acid was added to the pellet to prevent further metabolism. The samples were then separated by paper chromatography as described in the text. The results are expressed as the percentage of the total radioactivity present in either the AMP or the adenine peak.

radioactivity associated with the cell pellet reaching a peak at between 5 and 20min after addition of the adenine. The final concentration of the added adenine was 1 mM. After reaching a peak the cell-

associated radioactivity decreased to a value slightly below the zero-time value. In the case of AMP, uptake was slower reaching a peak at between 30 and 60min. Cell-associated radioactivity had decreased to a value slightly below the initial value by 150min. The uptake of AMP was also measured at a final concentration of 1 mM. For comparison the uptake of [1-¹⁴C]glycine by the same cells is shown in Fig. 3.

The uptake experiments were carried out with the cells at a concentration of $50 \times 10^6/\text{ml}$. Cell-volume measurements, as described in the Materials and Methods section, gave a hepatocyte volume of $1010 \pm 220 \mu\text{m}^3$; hence in these cell suspensions the cellular volume would be 5% of the volume of the suspension. If adenine and AMP were to equilibrate across the plasma membrane, the uptake should be of the order of 5%. This is in fact observed; as shown in Fig. 3 approx. 5% of adenine, AMP and glycine is associated with the cell pellets at their peak values. This may possibly be a fortuitous result, as the method used for measuring uptake measures only cell-associated radioactivity and does not distinguish between adsorption on to the cell surface and actual uptake into the intracellular volume.

The reason for the decrease in cell-associated adenine and AMP after the peak value had been reached is not known. This decrease does not appear to be an artifact of the method used, as glycine, under comparable conditions, is taken up and the cell-associated radioactivity is then constant at a plateau value. In the uptake experiments the total radioactivity of the suspension was constant during the whole incubation. The metabolism of the

added adenine and AMP was measured as indicated in Fig. 4. At different times during the course of uptake of adenine and AMP, samples of cell suspension were centrifuged (500g for 3 min) through Ficoll and the pellet was inactivated by the addition of 0.5M-formic acid. The suspension was chromatographed by using the solvent employed for the separation of adenine and AMP in the 5-phosphoribosyl 1-pyrophosphate assay (see the Materials and Methods section). In the adenine-uptake experiments, one peak of radioactivity in the cell pellet running with an adenine standard was observed during the time-course of the experiment. With AMP uptake, the AMP peak rapidly decreased and had disappeared after 60min (Fig. 4). In its place several other radioactive peaks of material appeared. These were not identified; however, one large peak of material progressively increased during the time-course of the experiment and ran with an adenine standard. Hence, although the final metabolite of AMP could not be identified unequivocally it was quite possibly adenine.

Intracellular 5-phosphoribosyl 1-pyrophosphate concentrations during incubation of liver cells

The 5-phosphoribosyl 1-pyrophosphate concentration of liver cells during incubation at 40°C was measured and the effect of AMP and adenine on this investigated. As shown in Fig. 5 the intracellular concentration remained relatively constant or decreased slightly during a 240min incubation at 40°C (within the accuracy of the assay, $\pm 17\%$). Whether 5-phosphoribosyl 1-pyrophosphate was measured in the total cell suspension or just in the cell pellet, after rapid centrifugation through Ficoll solution, the same values were obtained. This indicates that the 5-phosphoribosyl 1-pyrophosphate is entirely intracellular and does not diffuse out of the cells, or if it does it is not stable in the extracellular medium. The concentration of 5-phosphoribosyl 1-pyrophosphate was 7 ± 1 (3) nmol per 1×10^7 cells; with a cell volume of $1000 \mu\text{m}^3$ this gives an intracellular concentration of 0.7 ± 0.1 (3) mM. There was no measurable change in intracellular concentration of 5-phosphoribosyl 1-pyrophosphate on addition of AMP or adenine to a final concentration of 1 mM, over 3h.

5-Phosphoribosyl 1-pyrophosphate added to a final concentration of 1.8 mM did not alter the intracellular 5-phosphoribosyl 1-pyrophosphate concentration over 2h. A final concentration of 1.8 mM, assuming a cell volume of 5% of the total suspension volume and an initial endogenous intracellular 5-phosphoribosyl 1-pyrophosphate concentration of 0.7 mM, should lead to a 2.5-fold increase in intracellular concentration if 5-phosphoribosyl 1-pyrophosphate equilibrated across the plasma membrane. The lack of uptake of 5-phosphoribosyl 1-pyro-

Fig. 5. Intracellular 5-phosphoribosyl 1-pyrophosphate concentrations in liver cells during incubation at 40°C

Cells (10ml, at a concentration of $50 \times 10^6/\text{ml}$) were incubated at 40°C, 200 μl samples were removed and rapidly centrifuged through a 5% (w/v) Ficoll solution to separate the cell pellet. 5-Phosphoribosyl 1-pyrophosphate concentrations in the cell pellets were assayed as described in the text. The results of three individual experiments are shown.

phosphate, together with the finding that all the endogenous 5-phosphoribosyl 1-pyrophosphate in the cell suspension was intracellular, indicates that 5-phosphoribosyl 1-pyrophosphate does not cross the plasma membrane of liver cells. We have previously found (Badenoch-Jones & Buttery, 1975a) that addition of 1 mM-5-phosphoribosyl 1-pyrophosphate to liver cells did not affect total urate production, although previous reports from this laboratory showed that 5-phosphoribosyl 1-pyrophosphate stimulated total urate production in the perfused chick liver (Barratt *et al.*, 1974).

Discussion

The stimulatory effects of AMP and GMP on incorporation of glycine and formate into urate reported here are different from the previously reported inhibition of incorporation into liver but not into kidney cells (Badenoch-Jones & Buttery, 1975a). Although the reason for the different behaviour is not known, it seems to be a consequence of the different suspension media used. There could be a number of explanations as to why this

should be so. In the present work Eagle's minimum essential medium, containing an essential amino acid source, was used. Possibly in this case the amino acids are available for use as substrates for urate synthesis, whereas with a simple balanced salt solution, as used previously (Badenoch-Jones & Buttery, 1975a), substrates for urate synthesis come from catabolism of cellular proteins. In addition, in a medium containing amino acids, the cells are possibly actively synthesizing proteins, purines and pyrimidines in preparation for cell division. Hence cellular nitrogen metabolism may be different in the two media and thus may react to modifying agents, such as purines, in different ways. The use of a medium containing an amino acid source should be more 'physiological', and the cellular metabolism, under these conditions, should mirror metabolism *in vivo* in the intact tissue more closely. In the previous study of urate synthesis *de novo* in chick liver and kidney cells the incubation medium was Hanks' medium to which insulin was added (Badenoch-Jones & Buttery, 1975a). In the present work insulin did not affect the results obtained.

In mammalian cells, it is thought that purine synthesis *de novo* is rigidly controlled. Previously, on the basis of work on purified enzymes (see Buchanan, 1973) and ascites-tumour cells (Henderson, 1962), it was thought that feedback inhibition by purine products to the first committed enzyme of the purine-biosynthetic pathway (amidophosphoribosyltransferase, EC 2.4.2.14) was of prime importance in the regulation of the rate of purine synthesis. Recent work, especially on ascites-tumour cells, has concentrated on the intracellular concentration of 5-phosphoribosyl 1-pyrophosphate being rate-limiting for purine synthesis (Bagnara *et al.*, 1974; Henderson *et al.*, 1975) in intact cells. Indeed, it has been suggested that in intact ascites-tumour cells, feedback inhibition is inoperative (Henderson *et al.*, 1975). In lymphocytes evidence has been presented that intracellular 5-phosphoribosyl 1-pyrophosphate concentrations are rate-limiting for purine synthesis and increase markedly before an accelerated purine synthesis during mitogen-induced lymphocyte activation (Chambers *et al.*, 1974; Hovi *et al.*, 1975). It should be remembered, however, that the control of purine synthesis may be abnormal in tumour cells. In addition, the sudden increase in purine synthesis that occurs during lymphocyte activation is a special phenomenon.

Some of the work on inhibition of purified amidophosphoribosyltransferase has shown an inhibition by purine compounds of the avian enzyme (see Buchanan, 1973). The significance of this in the control of purine and urate synthesis in the chick is not clear, as a convincing case for the need for metabolic control of urate synthesis has not been proposed.

In the chick, urate is the main nitrogenous excretory product, and purine base and urate are thought to be synthesized by a common pathway through to inosinic acid (Hartman, 1970). It might be expected that neither feedback inhibition via the amidophosphoribosyltransferase nor the rate-limiting availability of 5-phosphoribosyl 1-pyrophosphate would be of importance. It may well be that the rate at which the pathway operates is dependent on the supply of excess of nitrogen, in the form of glutamine or ammonia, and that some inosinic acid is diverted to the synthesis of the purine bases. The control of purine-base synthesis is then likely to be exerted on the pathway between inosinic acid and the bases.

This does not, however, explain the observed effects of the addition of AMP, GMP, adenine and guanine on the incorporation of glycine or formate into urate. It is apparent that at least the incorporation of radioactively labelled substrate into urate is responsive to the intracellular concentrations of various purine compounds.

The addition of 1 mM concentrations of purine compounds may be regarded as unphysiologically high. However, this will depend on the uptake and subsequent metabolism of the added purines. There is also scant information on the normal intracellular concentrations of these purine compounds in chick liver. Hence intracellular concentrations of the various purine compounds do not necessarily mirror the added concentrations.

In this context, the results obtained in the present work are of interest. As shown in Fig. 3, although adenine is initially taken up by liver cells it is subsequently rapidly released. This means that between 60 and 240 min of incubation, when cells incubated with adenine show a decreased incorporation of glycine or formate into urate, the additional intracellular adenine concentration is negligible (Fig. 2). Similarly, at a time when cells incubated with AMP show an enhanced incorporation into urate, the AMP has been completely metabolized and the cell-associated concentration of AMP metabolites is negligible. The mechanism by which adenine and AMP metabolites are released from the cell is not known, although certainly in the case of adenine this must be released from the cells against a concentration gradient.

The inhibitory effect of adenine and guanine on the incorporation of formate or glycine into urate, adenine or guanine is not very marked, a 1 mM concentration of adenine or guanine being required to inhibit this incorporation by 50%. This compares with inhibition of purine synthesis (measured by the incorporation of [1-¹⁴C]glycine into formylglycine amide ribonucleotide in azaserine-blocked cells) of 90 and 40% in the presence of added adenine at a final concentration of 0.1 and 0.01 mM respectively, in intact ascites-tumour cells (Henderson,

1962). This may be related to the fact that there appears to be a mechanism in chick liver cells for keeping intracellular adenine low. The stimulation of incorporation with AMP and GMP is quantitatively larger, there being a 105% stimulation after 3 h at 1 mM. Inosine and inosinic acid also stimulate incorporation by the same order of magnitude; both of these compounds can be converted into AMP by chick liver (Hartman, 1970). 4-Amino-5-imidazolecarboxamide ribonucleoside inhibited the incorporation, a result that compares with an 4-amino-5-imidazolecarboxamide ribonucleoside-induced decrease in the incorporation of [1-¹⁴C]-glycine into formylglycine amide ribonucleotide in intact ascites-tumour cells (Henderson, 1962). 4-Amino-5-imidazolecarboxamide ribonucleoside is rapidly converted into urate by liver cells (Badenoch-Jones & Buttery 1975b). Hence it appears that the addition of compounds known to be rapidly converted into urate does not automatically lead to an increased incorporation of [1-¹⁴C]glycine into urate. It is noteworthy that the nucleotides AMP, GMP and IMP all stimulate the incorporation of glycine or formate into urate, whereas the bases adenine and guanine inhibit this incorporation.

The stimulatory effect of AMP or GMP and inhibitory effect of adenine or guanine is not explained by their altering the intracellular concentration of 5-phosphoribosyl 1-pyrophosphate. This is in contrast with the situation in ascites-tumour cells, where incubation with adenine or guanine up to 100 μ M lowers the intracellular 5-phosphoribosyl 1-pyrophosphate concentration as these compounds are converted into AMP and GMP by a 5-phosphoribosyl 1-pyrophosphate-dependent reaction (Bagnara *et al.*, 1974). The enzyme converting adenine into AMP, adenine phosphoribosyltransferase, is certainly present in chick liver and is active in Eagle's medium, as this is used for the 5-phosphoribosyl 1-pyrophosphate assay (see the Materials and Methods section). However, the results obtained in the present work (Fig. 4) show that adenine taken up by the cell is not metabolized to AMP in detectable quantities.

That lymphoid cells incorporate glycine and formate into urate at such a high rate is perhaps fortuitous, lymphoid tissue being primarily concerned with immunological responses. Lymphocytes certainly have the ability to synthesize purines *de novo* (Hovi *et al.*, 1975; Chambers *et al.*, 1974). It may well be that they also possess a degradative pathway capable of converting preformed bases into urate and hence the added glycine or

formate may finally be incorporated into urate under the incubation conditions used. This would be an alternative to glycine or formate being incorporated directly into urate.

Cells from other chick tissues also possessing these pathways may well incorporate glycine and formate into urate. In the case of the bursa cells it is not clear why they should incorporate added glycine and formate into urate so much faster than do the other cell types. This may not indicate a faster actual rate of synthesis of urate. It is also possible that some other cell type, other than lymphocytes, may be responsible for urate production.

The bursa is connected directly to the cloaca of the chick (see Payne, 1971) and thus would be ideally situated to discharge newly synthesized urate directly into the cloaca. However, whether this is a specific function of the bursa remains to be seen.

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