Characteristics of Aspartate Deamination by the Purine Nucleotide Cycle in the Cytosol Fraction of Rat Liver

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1. The component reactions of the purine nucleotide cycle were studied in cytosol extracts of rat liver. 2. AMP deaminase was strongly activated by ATP and analogues of ATP. 3. IMP was converted into ATP by a system requiring the presence of aspartate, GTP and a nucleoside triphosphate-regenerating system. 4. Under appropriate conditions, NH_3 was produced from aspartate. 5. From the rates at which these reactions occur it is concluded that the purine nucleotide cycle may have sufficient activity to be a major pathway of amino acid deamination in liver.

The pathway of urea synthesis from amino acids in liver involves the initial deamination of amino acids to form NH_3 , followed by the subsequent conversion of NH_3 into urea by the enzymes of the urea cycle. Amino acid deamination has been considered to proceed mainly via the 'trans-deamination' pathway (see Braunstein, 1957). The amino acid nitrogen is transferred to 2-oxoglutarate via a number of specific transaminases with the formation of glutamate. Glutamate is then deaminated by reaction with glutamate dehydrogenase in the mitochondria.

It has been shown that L-leucine activates glutamate dehydrogenase in sonicated and intact liver mitochondria (McGivan *et al.*, 1973), but inhibits urea synthesis from amino acids in isolated liver cells (Mendes-Mourão *et al.*, 1975). These results have been interpreted to indicate that glutamate dehydrogenase may have less importance in ammoniogenesis in liver than has been considered to be the case (Mendes-Mourão *et al.*, 1975; McGivan & Chappell, 1975). It is therefore of importance to determine whether any other general pathway of amino acid deamination has sufficient activity to account for the rate of urea synthesis from amino acids observed in perfused liver or in isolated liver cells.

It has been recognized for some time that the production of NH_3 from AMP via AMP deaminase and the subsequent reamination of IMP by the amino group of aspartate could constitute an alternative pathway of amino acid deamination (see Braunstein, 1957). This pathway [which has been termed the 'purine nucleotide cycle' (Lowenstein, 1972)] has been shown to have high activity in extracts of rat skeletal muscle (Tornheim & Lowenstein, 1972; Lowenstein, 1972). It was considered that the purine nucleotide cycle was unlikely to be of major importance in ammoniogenesis in liver, since the apparent activity of AMP deaminase

observed in liver extracts was very low compared with that of glutamate dehydrogenase.

The present paper describes the characteristics of the component reactions of the purine nucleotide cycle in cytosol extracts of rat liver. It is concluded that this pathway may be of major importance in the production of NH_3 from aspartate and hence from other amino acids.

Experimental

Preparation of a cytosol fraction from rat liver

Female Wistar rats weighing approx. 200g were starved for 48h before use. Rats were killed by decapitation and the liver from each rat was chopped and homogenized in a Dounce homogenizer with a Teflon pestle of 0.3mm clearance in 40ml of a medium containing 0.15M-KCl, 0.01M-MgCl₂, 0.1mM-dithiothreitol and 0.01M-3-(N-morpholino)propanesulphonic acid at pH7.0 and 0°C. The homogenate was centrifuged at 1000g for 10min at 4°C to sediment cell debris, nuclei and some mitochondria. The supernatant was then centrifuged at 38000g for 30min to remove the remaining mitochondria and microsomal fraction. The use of the preparation medium described above ensured minimum microsomal contamination and provided conditions for maximum activity of AMP deaminase, which is activated by alkali-metal cations (Smith & Kizer, 1969).

The supernatant from the final centrifugation was applied in 5ml portions to columns (2cm×15cm) of Sephadex G-25 (coarse grade) and eluted with the above medium at 4°C to remove endogenous substrates. Fractions were collected, and those richest in protein were pooled. The final suspension, containing 10–15mg of protein/ml, was used as the cytosol fraction of rat liver.

Incubations

Cytosol extract prepared as described above was adjusted to pH7 with KOH or HCl and incubated with shaking at 37°C. The reaction was started by the addition of substrates in very small volumes. At intervals samples were removed and deproteinized with HClO₄ (final concentration 5%, w/v). After removal of the precipitate by centrifugation at 10000g for 2min the samples were neutralized with KOH and cooled to 0°C to ensure complete precipitation of KClO₄. The KClO₄ was removed by centrifugation and the samples were used for assays of metabolites.

Assays

NH₃ (Kirsten *et al.*, 1963), aspartate (Pfleiderer, 1965), ATP (Lamprecht & Trautschold, 1965), ADP and AMP (Adam, 1965) were assayed enzymically. Malate was assayed by using malate dehydrogenase (Hohorst, 1965) and fumarate was determined in the same sample by the addition of fumarase after the malate assay had proceeded to completion.

The activity of lactate dehydrogenase was determined as described by Bergmeyer *et al.* (1965) and that of glucose 6-phosphate dehydrogenase as described by Lohr & Waller (1965). Adenylate kinase was assayed as follows. In the case of the cytosol extract prepared in the presence of MgCl₂, cytosol extract (approx. 0.1mg of protein) was incubated in 2.5ml of a medium containing 100mM-KCl, 10mM-3-(*N*-morpholino)propanesulphonic acid, 10mM-glucose, 10mM-MgCl₂, 0.5mM-NADP⁺, 10μg of hexokinase and 10μg of glucose 6-phosphate dehydrogenase at pH7.0 and 37°C. The reaction was started by the addition of 2mM-ADP and the rate of NADP⁺ reduction was followed at 340nm. When cytosol extract was prepared in the absence of MgCl₂, the assay was performed by incubating the extract (1–5mg) in 4ml of medium containing 100mM-KCl and 10mM-3-(*N*-morpholino)propanesulphonic acid at pH7.0 and 37°C; 2mM-ADP was added. Samples were withdrawn after 1, 2 and 3 min, deproteinized and neutralized as indicated above and assayed for ATP by the method of Lamprecht & Trautschold (1965).

5'-Nucleotidase, glucose 6-phosphatase and adenosine triphosphatase were assayed as follows. Cytosol extract (approx. 10mg of protein/ml) was incubated at pH7.0 and 37°C. The reaction was started by the addition of the appropriate substrate at a concentration of 2mM. Samples were withdrawn after 0, 5 and 10min, deproteinized and assayed for P_i.

Glutamate dehydrogenase was assayed by the method of Schmidt (1965).

P_i was determined by a modification of the method of Berenblum & Chain (1938). Protein was determined by a biuret method (Gornall *et al.*, 1949).

Materials

Enzymes, adenine and nicotinamide nucleotides and adenylyl imidodiphosphate were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Solid hexokinase was purchased from BDH Chemicals Ltd., Poole, Dorset, U.K. Adenylyl-(βγ-methylene)-diphosphonate was obtained from Miles Laboratories Inc., Elkhart, Ind., U.S.A.

Results

In extracts of rat skeletal muscle, aspartate is deaminated to form fumarate+NH₃ by the following sequence of reactions, known as the purine nucleotide cycle (Tornheim & Lowenstein, 1972):

Net reaction:

The results presented below show the characteristics of the deamination of AMP and of the conversion of IMP into adenine nucleotides in cytosol extracts of rat liver.

Deamination of AMP

In cytosol extracts of rat liver, NH₃ production from AMP may occur either directly by the action of AMP deaminase (EC 3.5.4.6) or indirectly by the initial hydrolysis of AMP to adenosine catalysed by 5'-nucleotidase (EC 3.1.3.5) followed by deamination of adenosine via adenosine deaminase (EC 3.5.4.4) (see Kizer *et al.*, 1963).

Table 1 shows the activity of certain enzymic reactions in cytosol extracts as prepared. The following points may be noted. In the presence of Mg²⁺ the extract contained a high adenylate kinase activity. Both AMP and IMP were hydrolysed by 5'-nucleotidase at a significant rate. The extracts contained some adenosine triphosphatase activity; however, when both AMP and ATP were present simultaneously, the rate of phosphate production was considerably less than the sum of the rates obtained when these compounds were present separately. This finding indicates that 5'-nucleotidase is strongly inhibited by ATP, as in rat heart (Baer *et al.*, 1966). The cytosol extract contained some adenosine deaminase activity. Microsomal contamination was low, as judged by the activity of

Table 1. Rates of some enzymic reactions in cytosol extracts of rat liver

The rates of the reactions, determined as described in the Experimental section, were linear with time over the first 10 min, except for adenylate kinase. Adenylate kinase was measured continuously in the presence of Mg^{2+} , and by stopped assay in the absence of Mg^{2+} . The rates quoted are the means of duplicate determinations performed on two separate preparations of cytosol extract. In determinations performed in the absence of Mg^{2+} , the $MgCl_2$ in the preparation medium was replaced by 1 mM-EDTA.

Enzyme reaction	Substrate	Rate (nmol/min per mg)	
		Mg^{2+} present	Mg^{2+} absent
Adenylate kinase	2 mM-ADP	125	1
5'-Nucleotidase	2 mM-AMP	4.0	1.2
5'-Nucleotidase	2 mM-IMP	2.7	0.4
ATP hydrolysis	1 mM-ATP	2.6	0.9
5'-Nucleotidase plus ATP hydrolysis	2 mM-AMP + 1 mM-ATP	3.2	1.0
Adenosine deaminase	2 mM-Adenosine	6.3	—
Glucose 6-phosphatase	5 mM-Glucose 6-phosphate	0.7	1.9

Fig. 1. AMP deamination in Mg^{2+} -free cytosol extracts

The cytosol extract was prepared as described in the Experimental section, except that $MgCl_2$ in the preparation medium was replaced with 1 mM-EDTA. The rate of NH_3 production was linear with time over the first 5 min of the incubation. The rate of NH_3 production from 1 mM-ATP in the absence of AMP was negligible. ●, +1 mM-ATP; ○, ATP absent.

glucose 6-phosphatase. The activity of glucose 6-phosphatase recovered in the total cytosol fraction was approx. 2% of that observed in whole homogenates on the basis of previously published values (Song & Bodansky, 1967). In the absence of Mg^{2+} , the activity of adenylate kinase was very low and the activities of 5'-nucleotidase and adenosine triphosphatase were also much decreased. Accordingly, the deamination of AMP was initially studied in extracts containing no $MgCl_2$.

Fig. 2. Dependence of the rate of AMP deamination on the concentration of ATP in the absence of Mg^{2+}

The conditions of the incubation were those described in the legend to Fig. 1. The AMP concentration was 2 mM. The rate of NH_3 production from 5 mM-ATP in the absence of AMP was 1.7 nmol/min per mg.

Fig. 1 shows that the rate of deamination of AMP was relatively low in the absence of ATP and that the addition of 1 mM-ATP caused a very marked activation. The NH_3 production observed was due almost entirely to the action of AMP deaminase, since the corresponding rate of P_i production from AMP was very low and was inhibited strongly by ATP (Table 1). It is noteworthy that AMP deaminase was markedly activated by ATP even at low AMP concentrations. The concentration-dependence of the activation by ATP is shown in Fig. 2. Half-maximum stimulation was observed with 0.25 mM-ATP; 1 mM-ATP caused a sixfold stimulation

Fig. 3. Activation of AMP deaminase by analogues of ATP in the presence of Mg^{2+}

Cytosol extract was prepared as described in the Experimental section. The reaction was started by the addition of 2mM-AMP in the presence of ATP analogues as indicated. The rate of NH_3 production from the analogues in the absence of AMP was negligible. ●, +Adenylyl imidodiphosphate; ○, +adenylyl-(β,γ -methylene)diphosphonate.

of AMP deaminase activity when 2mM-AMP was present. These results are consistent with those of other authors, who have shown that AMP deaminases isolated from rat liver (Smith & Kizer, 1969) and from calf brain (Setlow & Lowenstein, 1968) are activated by ATP.

AMP deamination was strongly activated by ATP also in the presence of Mg^{2+} (results not shown). No quantitative interpretation could be placed on this finding, since in the absence of ATP there was a relatively high 5'-nucleotidase activity, whereas in the presence of ATP, adenylate kinase catalysed the rapid formation of ADP from ATP+AMP (Table 1). It could be shown that AMP deaminase activity was high also in the presence of Mg^{2+} by the use of analogues of ATP to activate the enzyme. Fig. 3 shows that the production of NH_3 from AMP was stimulated by both adenylyl-imidodiphosphate and adenylyl-(β,γ -methylene)diphosphonate. Again, the NH_3 production was due very largely to the action of AMP deaminase. It was shown that the ATP analogues markedly inhibited the rate of phosphate release from AMP. Activation by adenylyl-(β,γ -

Table 2. Requirements for the synthesis of ATP from IMP

Additions to the cytosol extract were 5mM-aspartate, 1mM-IMP, 0.5mM-GTP, 5mM-creatine phosphate and 2.5 units of creatine kinase/ml as indicated. Samples were withdrawn for assay after 7.5 and 15 min.

Additions	Rate of ATP synthesis (nmol/min per mg)
Complete system	1.96
Complete system—aspartate	0.07
Complete system—IMP	<0.05
Complete system—GTP	0.15
Complete system—creatine phosphate	<0.05
Complete system—creatine kinase	0.95
None	<0.05

methylene)diphosphonate of AMP deaminase isolated from calf brain has been reported by Setlow & Lowenstein (1968).

Formation of adenine nucleotides from IMP plus aspartate

To demonstrate the conversion of IMP into AMP by reactions (1) and (2) above, it was necessary to provide a system for the regeneration of GTP from GDP and phosphate, since adenylosuccinate synthetase (EC 6.3.4.4) is strongly inhibited by GDP (Muirhead & Bishop, 1974). This was done by the use of creatine phosphate plus creatine kinase. This system converts both ADP and GDP into the corresponding triphosphates. Consequently, any AMP formed was rapidly converted into ATP by the combined actions of endogenous adenylate kinase plus the added creatine kinase. The reaction was probably initiated by GTP via a nucleoside triphosphate-AMP phosphotransferase.

Table 2 shows that the synthesis of ATP from IMP has an absolute requirement for aspartate, GTP, IMP and creatine phosphate. Only a partial requirement for added creatine kinase was indicated, suggesting that the liver extract contained some endogenous creatine kinase activity. The time-course of ATP synthesis is shown in Fig. 4. ATP synthesis and aspartate disappearance were linear with time for periods up to 30 min. Aspartate disappearance was accompanied by the appearance of malate in the incubation. This was to be expected, since the cytosol extract contained a high fumarase activity, and the equilibrium of the fumarase reaction favours malate formation. The removal of AMP by adenylate kinase and creatine kinase prevented AMP deamination. Adenylosuccinate was not determined, since no appropriate assay was available. The cessation of the reaction after 30 min was presumably due to the exhaustion of the added IMP which, in addition to its

reaction with aspartate via adenylosuccinate synthetase, was also hydrolysed to inosine by 5'-nucleotidase (Table 1), at least in the absence of ATP.

Fig. 5 shows the dependence of the rate of ATP synthesis on the initial concentrations of aspartate and GTP. The apparent K_m for aspartate was 0.6 mM,

Fig. 4. Time-course of synthesis of ATP from IMP + aspartate

The reaction was started by the addition to the cytosol extract of the following components: 2 mM-aspartate, 1 mM-IMP, 0.5 mM-GTP, 5 mM-creatine phosphate and 2.5 units of creatine kinase/ml. The concentrations of AMP and fumarate were less than 1.5 nmol/mg throughout. □, Aspartate; ○, ATP; ●, malate; ▲, NH₃.

and that for GTP was 0.35 mM. No meaningful K_m value for IMP could be obtained, owing to some hydrolysis of IMP by 5'-nucleotidase. It was consistently found that the rate of ATP synthesis at 2 mM-IMP was not significantly different from that obtained at 1 mM-IMP. The maximum rate of ATP synthesis from 5 mM-aspartate + 0.5 mM-GTP + 1 mM-IMP in eleven separate preparations was 2.1 ± 0.3 (s.e.m.) nmol/min per mg of protein. This rate was not stimulated by the addition of 2 mM-potassium phosphate, 5 mM-ornithine, 5 mM-glutamate or 5 mM-glutamine.

Deamination of aspartate

To demonstrate the production of NH₃ from aspartate via AMP deaminase, it was necessary to add a specific ATP-hydrolysing system to allow the accumulation of AMP. Table 3 depicts the results of such an experiment. The cytosol extract was incubated with IMP, GTP, aspartate, creatine phosphate and creatine kinase as before, and glucose and hexokinase were also present initially. The aspartate nitrogen disappearance was largely accounted for by the initial accumulation of AMP and the subsequent formation of NH₃. It is noteworthy that the rate of NH₃ production was relatively slow. This was to be expected, since no ATP was present and AMP deaminase was thus not activated.

Fig. 6 shows the results of a similar experiment in which glucose and hexokinase were added after the aspartate nitrogen had been transferred to ATP. Under these conditions, NH₃ was liberated from

Fig. 5. Dependence of the initial rate of ATP production on the concentrations of (a) aspartate and (b) GTP

The reaction was started by the addition to the cytosol extract of 1 mM-IMP, 5 mM-creatine phosphate and 2.5 units of creatine kinase/ml together with in (a) 0.5 mM-GTP and various concentrations of aspartate and in (b) 5 mM-aspartate and various concentrations of GTP. Samples were deproteinized after 7.5 and 15 min.

Table 3. *Production of NH₃ from aspartate*

The reaction was started by the addition to the extract of 2mM-aspartate, 1mM-IMP, 0.5mM-GTP, 5mM-creatine phosphate, 2.5 units of creatine kinase/ml, 10mM-glucose and 0.1 mg of hexokinase/ml. No significant concentration of ATP was found in the extract.

Time min	Aspartate disappearance (nmol/ng)	Metabolite (nmol/mg)		
		Malate	AMP	NH ₃
5	5.5	3.0	0	0
10	9.0	5.7	0.8	0.8
15	11.5	8.0	6.0	2.8
20	13.8	9.6	5.6	5.2
25	15.0	10.9	3.4	8.0
30	15.8	11.6	2.0	10.5
40	16.8	12.5	0	15.0

Fig. 6. *Production of NH₃ from aspartate*

The reaction was started by the addition to the cytosol extract of 2mM-aspartate, 1mM-IMP, 0.5mM-GTP, 5mM-creatine phosphate and 2.5 units of creatine kinase/ml. After 25 min, 10mM-glucose and 0.1 mg of hexokinase/ml were added. □, Aspartate; ○, ATP; ▲, NH₃.

ATP by the combined actions of hexokinase, adenylate kinase and AMP deaminase (see also Kun *et al.*, 1966). Again, under these conditions, the activity of AMP deaminase was limited by the absence of ATP. It was also likely that a certain

amount of AMP was deaminated indirectly by the action of 5'-nucleotidase and adenosine deaminase after the ATP concentration had fallen.

Conversion of reaction rates expressed per mg of cytosol protein into corresponding rates expressed per g wet wt. of liver

The cytosol extract was characterized by assaying the activity of lactate dehydrogenase and glucose 6-phosphate dehydrogenase in the liver homogenate and in the cytosol extract. These two enzymes are considered to be located exclusively in the cytosol. This procedure was necessary since the homogenization did not break all the liver cells. In five separate preparations, the total activity of lactate dehydrogenase in the homogenate solubilized by the addition of Triton X-100 (final concentration 0.1%) was 912 ± 104 (s.e.m.) $\mu\text{mol/min per g wet wt.}$ at 37°C and that of glucose 6-phosphate dehydrogenase was $12.6 \pm 2.4 \mu\text{mol/min per g}$. The corresponding activities in the cytosol extract were $6.8 \pm 1.4 \mu\text{mol/min per mg of cytosol protein}$ and $0.076 \pm 0.016 \mu\text{mol/min per mg of cytosol protein}$ respectively. Rates expressed per mg of cytosol protein may thus be converted into corresponding rates per g wet wt. by multiplying by 142 (based on lactate dehydrogenase assays) or by 173 (based on glucose 6-phosphate dehydrogenase assays). In practice, a mean conversion factor of 158 was used in the calculations shown in Table 4 and discussed below.

Possible mitochondrial breakage was assessed by determination of glutamate dehydrogenase activity in the extract at 37°C and pH 7.0. A rate of $1.1 \pm 0.2 \text{ nmol/min per mg of cytosol protein}$ was obtained, which corresponds to $0.16 \mu\text{mol/min per g of liver}$. The total glutamate dehydrogenase activity is approx. $250 \mu\text{mol/min per g of liver}$ (see Lowenstein, 1972).

Discussion

Characteristics of the component reactions of the purine nucleotide cycle

The results presented above show that in cytosol extract of rat liver the amino group of aspartate can be donated to IMP with the formation of AMP; the amino group is then liberated as NH₃ by the action of AMP deaminase. AMP deaminase in liver extracts is very markedly activated by ATP. It appears that at levels of ATP that have been measured in intact liver (Woods *et al.*, 1970) this enzyme would always be maximally activated (Fig. 2). Thus the activation by ATP is unlikely to be an important control mechanism for AMP deamination *in vivo*. The actual rate of deamination of AMP observed in the presence of ATP was critically dependent on the concentration of AMP at low AMP concentrations (Fig. 1).

The apparent K_m for aspartate of the synthesis of ATP from IMP plus aspartate was 0.6mM (Fig. 5). This value is comparable with the levels of aspartate that occur in freeze-clamped liver *in vivo* (Williamson *et al.*, 1967). Under the conditions used in the present investigation, the rate of formation of ATP from IMP plus aspartate was 2.1nmol/min per mg. However, it is unlikely that the maximum rate at which amination of IMP may occur *in vivo* was measured under these conditions. In particular, in cytosol extracts as prepared, there was a considerable rate of hydrolysis of IMP by 5'-nucleotidase in the absence of ATP (Table 1). It is also possible that partial inactivation of adenylosuccinate synthetase or of adenylosuccinase (EC 4.3.2.2) occurred owing to dilution during preparation of the extract or that an essential activator was absent.

Operation of the purine nucleotide cycle in the liver cell

It has been shown that NH_3 can be produced from aspartate in cytosol extracts. However, it was not possible to demonstrate the continuous production of NH_3 by the efficient operation of the entire purine nucleotide cycle for the following reasons.

(i) No NH_3 is produced in the absence of an added adenosine triphosphatase, since the AMP concentration is kept too low (Fig. 4).

(ii) In the presence of excess of adenosine triphosphatase activity, no ATP is present to activate AMP deaminase (Table 3).

(iii) In the absence of ATP, IMP is hydrolysed to inosine (and AMP is to some extent hydrolysed to adenosine; Table 1), thus preventing the recycling of IMP formed by AMP deaminase.

(iv) There is a limitation imposed by the amount of the nucleoside triphosphate-regenerating system added.

It was not possible to poise the activities of ATP synthesis and ATP hydrolysis at the appropriate values to ensure continuous operation of the cycle in cytosol extracts.

In perfused liver, the relative activities of ATP synthesis by mitochondria, of ATP hydrolysis by cytoplasmic reactions and of adenylate kinase allow the existence of a considerable intracellular level of AMP in the presence of a much higher level of ATP (Woods *et al.*, 1970). There is present also a certain amount of IMP (Woods *et al.*, 1970). The level of ATP present is sufficient to activate AMP deaminase

Table 4. Comparison of urea production in liver cells with the rate of certain processes in isolated mitochondria and in cytosol extracts of liver

The values quoted are the mean $\pm$ s.e.m. for the number of separate preparations indicated in parentheses. The equivalent rates of urea synthesis per g wet wt. of liver were calculated on the basis that 1 g of liver is equivalent to 158 mg of cytosol protein under the conditions used (see the text), to 40 mg of mitochondrial protein, or to 140 mg of liver cell protein.

System	Parameter measured	Rate (nmol/min per mg)	Reference	Equivalent rate urea synthesis ($\mu\text{mol/min per g}$ wet wt. at 37°C)
Isolated liver cells	Urea synthesis from 10 mM-alanine	2.76 ± 0.38 (6)	Mendes-Mourão <i>et al.</i> (1975)	0.38
Cytosol extract of rat liver	Production of ATP from aspartate	2.1 ± 0.3 (11)	The present paper	0.33
Cytosol extract of rat liver	Production of NH_3 from 0.2 mM-AMP by activated AMP deaminase	3.7	The present paper (Fig. 1)	0.59
Isolated mitochondria	Production of NH_3 from glutamate (20 mM) at 30°C under optimum conditions	4.3 ± 0.7 (4)	McGivan <i>et al.</i> (1974)	0.08*
Isolated mitochondria	Production of citrulline from glutamate (20 mM) at 30°C under optimum conditions	2.5 ± 0.2 (4)	McGivan <i>et al.</i> (1974)	0.04*
Isolated mitochondria	Production of citrulline from NH_3 (10 mM) at 30°C under optimum conditions	14.7 ± 1.0 (3)	N. M. Bradford (personal communication)	1.18†
Cytosol extract of rat liver	Production of urea from citrulline+aspartate	12.7 (2)	Mendes-Mourão <i>et al.</i> 1975	2.00

* This value is derived on the assumption that the rate-limiting glutamate transport system has a K_m for glutamate of 4mM (Bradford & McGivan, 1973) and that the concentration of glutamate in the cytosol is approx. 2mM. To correct for the difference in temperature, the rate is multiplied by 1.5 (Bradford & McGivan, 1973).

† The assumptions made are that the K_m of citrulline synthesis for NH_3 in mitochondria is 0.2mM (McGivan *et al.*, 1973) and that the intracellular concentration of NH_3 in liver is greater than 0.5mM. A factor of 2 is used to correct for the temperature difference.

maximally and to inhibit to a large extent the action of 5'-nucleotidase. Under these conditions it is to be expected that the purine nucleotide cycle would operate efficiently with the continuous deamination of AMP and subsequent re-amination of the IMP formed.

Comparison of the rates of NH₃ production obtainable from the purine nucleotide cycle and from glutamate dehydrogenase

Table 4 shows the rates of a number of metabolic processes that have been observed in isolated liver mitochondria and in cytosol extracts. These rates are extrapolated to give equivalent rates of urea synthesis on the basis of certain assumptions, which are stated in the legend to Table 4. The rates of citrulline production from NH₃ in isolated mitochondria and of urea synthesis from citrulline+ATP+aspartate in cytosol extracts are greatly in excess of the rate at which urea synthesis from alanine occurs in isolated liver cells. The rate of production of NH₃ from aspartate as judged by ATP synthesis from IMP+aspartate or by NH₃ production from physiological levels of AMP is of the magnitude required to account for urea synthesis from alanine. In contrast, the rates of glutamate deamination via glutamate dehydrogenase and of citrulline synthesis from glutamate in isolated mitochondria are much lower and are inadequate to account for urea synthesis from alanine. In intact mitochondria, glutamate deamination and citrulline synthesis from glutamate are rate-limited by the inward transport of glutamate across the mitochondrial inner membrane (Bradford & McGivan, 1973; McGivan *et al.*, 1974; McGivan & Chappell, 1975).

One reason for the contention that glutamate dehydrogenase is primarily responsible for the production of NH₃ from amino acids in liver is that its apparent activity in liver extracts (measured in the direction of glutamate synthesis) is much higher than that of AMP deaminase (see, Lowenstein, 1972). However, the present study has shown that the maximum rate of AMP deamination in the presence of 1 mM-ATP is in excess of 6 $\mu\text{mol/min per g wet wt. of liver}$, whereas the maximum rate of NH₃ production from glutamate as judged by glutamate deamination in intact mitochondria is not greater than 0.4 $\mu\text{mol/min per g of liver}$.

It is concluded that the purine nucleotide cycle in liver may well have sufficient activity under physiological conditions to be a major pathway of NH₃ production from aspartate and hence from other amino acids whose amino groups may be transferred to aspartate via glutamate. If the assumptions made in Table 4 are justified, it may be concluded that the rate of NH₃ production obtainable via the

purine nucleotide cycle in liver is considerably greater than the rate of NH₃ production that can be obtained from glutamate dehydrogenase.

Information about the turnover of the 6-NH₂ group of adenine in liver would be helpful in evaluating the physiological significance of the purine nucleotide cycle in NH₃ production.

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