The Effects of Ammonium Chloride and Bicarbonate on the Activity of Glutaminase in Isolated Liver Mitochondria

By SURESH K. JOSEPH and JOHN D. MCGIVAN

Department of Biochemistry, University of Bristol Medical School, University Walk, Bristol BS8 1TD, U.K.

(Received 29 June 1978)

1. Glutamine hydrolysis in liver mitochondria was studied by measuring the production of glutamate under conditions where this compound could not be further metabolized. 2. Glutaminase activity in intact mitochondria was very low in the absence of activators. 3. Glutamine hydrolysis was markedly stimulated by NH_4Cl and also by HCO_3^- ions. 4. The stimulation by each of these compounds was much decreased if the mitochondria were uncoupled. 5. Maximum rates of glutamine hydrolysis required the addition of phosphate. A correlation was observed between the activity of glutaminase in the presence of NH_4Cl plus HCO_3^- and the intramitochondrial content of ATP. 6. In disrupted mitochondria, NH_4Cl stimulated glutaminase to a much smaller extent than in intact mitochondria. The NH_4Cl stimulation in disrupted mitochondria was much increased by the addition of ATP. KHCO_3 also stimulated glutaminase activity in disrupted mitochondria, and ATP increased the magnitude of this stimulation. 7. It was concluded that maximum rates of glutaminase activity in liver mitochondria require the presence of phosphate, ATP and either HCO_3^- or NH_4^+ . A comparison of the results obtained on intact and broken mitochondria indicates that these effectors have a direct effect on the glutaminase enzyme system rather than an indirect effect mediated by changes in transmembrane ion gradients or in the concentrations of intramitochondrial metabolites.

The role of the liver in mammalian glutamine metabolism is somewhat uncertain. Liver extracts contain high activities of glutaminase and of glutamine synthetase. Measurements of blood glutamine concentrations indicate that the liver may catalyse either the net degradation or net synthesis of glutamine under different conditions (Ishikawa, 1975). Other results, however, suggest that the liver may not contribute significantly to glutamine metabolism (Lund & Watford, 1976). Perfused liver does not catalyse the net synthesis of glutamine to any extent (Lund, 1971). Although 10mM-glutamine is a good substrate for gluconeogenesis in isolated liver cells (Krebs *et al.*, 1976), glutamine at low concentrations is not metabolized (Lund & Watford, 1976).

In contrast with kidney glutaminase, the liver enzyme has not been intensively studied. In liver, glutaminase I (EC 3.5.1.2) is situated in the mitochondrial matrix (Kala & Brosnan, 1973). Glutamine is a poor respiratory substrate for liver mitochondria (Hird & Marginson, 1968). The glutamine-hydrolysing activity of liver mitochondria has been reported to be activated by *N*-acetylglutamate (Katunuma *et al.*, 1965; Blackburn *et al.*, 1972), phosphate (Blackburn *et al.*, 1972) and ammonia (Charles, 1968).

Recent work has shown that, in intact liver cells, glutamine degradation is specifically stimulated by

NH_4Cl and by the hormone glucagon (Joseph & McGivan, 1978). The present investigation was undertaken to obtain more information about the conditions necessary for the maximum activity of glutaminase in liver mitochondria.

Materials and Methods

Preparation and incubation of mitochondria

Mitochondria were prepared by the method of Chappell & Hansford (1972) from the livers of normally fed female Wistar rats weighing approx. 200g. Incubations were carried out at 37°C in a shaking water bath. Media were made up with double-distilled water that had been boiled just before use and kept under soda-lime to remove any dissolved CO_2 . Unless otherwise stated, all experiments were carried out at pH 7.3. When the incubation medium contained 20mM- KHCO_3 , it was bubbled before use with O_2/CO_2 (19:1) and the incubation was carried out in closed glass vessels under the same gas phase. When HCO_3^- was not added, the gas phase was O_2 alone.

Incubations were terminated by the addition of HClO_4 (3.5%, w/v, final concentration) to the mitochondrial suspension. After centrifugation (10000g for 2min) and removal of the precipitated protein,

the supernatant was neutralized with 3M-KOH and used for the assay of metabolites.

Measurement of glutaminase activity

Glutaminase activity is commonly measured by following the release of NH_3 from added glutamine. This method was not appropriate in the present investigation, since NH_4Cl was frequently added to activate the enzyme. Glutaminase activity was therefore assayed by measuring the production of glutamate from glutamine under conditions where the glutamate could not be further metabolized. When intact mitochondria were used rotenone was routinely added to prevent glutamate oxidation. Transamination of glutamate did not occur to any extent, since in the presence of rotenone no oxaloacetate could be produced. In disrupted mitochondria, glutamate oxidation cannot occur in the absence of added NAD^+ and transamination cannot occur in the absence of added oxaloacetate.

In control experiments, it was shown that in intact mitochondria rotenone completely inhibited

glutamate metabolism. In disrupted mitochondria under the conditions used, no metabolism of added glutamate occurred. It was also shown that the time course of glutamate production from glutamine was linear over a 10min period in disrupted mitochondria and was linear after an initial lag of 1–2min in intact mitochondria. Glutamate production during a 10min incubation was therefore taken as a measure of glutaminase activity. Corrections were always made for the amount of glutamate present at the start of the incubation. This was necessary since the glutamine used contained approx. 1% glutamate as an impurity.

Assays

Glutamate was assayed enzymically by the method of Bernt & Bergmeyer (1965). It was established that neither NH_4Cl nor KHCO_3 at the concentrations used interfered with this assay. Intramitochondrial ATP was assayed by using luciferase (Stanley & Williams, 1969). Mitochondrial protein was measured by using a biuret method (Gornall *et al.*, 1949).

Fig. 1. Effect of NH_4Cl , KHCO_3 and of the energy state on glutaminase activity in intact liver mitochondria. Mitochondria were incubated at a concentration of 3.2mg of protein/ml in a medium containing 120mM-KCl, 20mM-Tris/HCl, 5mM-potassium succinate, 10mM-potassium phosphate, 10 μg of rotenone/ml and various concentrations of glutamine at pH7.3 and 37°C. Other additions were 5mM- NH_4Cl , 20mM- KHCO_3 and 0.5 μM -carbonyl cyanide *m*-chlorophenylhydrazine (Cl-CCP) as indicated. Glutamate production was measured after 10min. Symbols: (a): ▲, no additions; ●, + NH_4Cl ; ○, + NH_4Cl +Cl-CCP. (b): ●, + KHCO_3 ; ○, + KHCO_3 +Cl-CCP. (c): ●, + KHCO_3 + NH_4Cl ; ○, + KHCO_3 + NH_4Cl +Cl-CCP.

Results

Glutaminase activity in intact mitochondria

Mitochondria were incubated with succinate plus phosphate in the presence of rotenone to inhibit the metabolism of glutamate. Under these conditions glutaminase activity was negligible over a 10 min interval at 37°C. Fig. 1(a) shows that glutamine hydrolysis was very markedly stimulated by the addition of 5 mM-NH₄Cl, in accordance with previous observations (Charles, 1968; Joseph & McGivan, 1978). Glutamine hydrolysis was also stimulated to a similar extent by the addition of 20 mM-KHCO₃ (Fig. 1b). Fig. 1(c) shows that the activation observed when KHCO₃ and NH₄Cl were added together was not much greater than when these were added separately. Fig. 1 demonstrates that the stimulation of glutaminase activity by both NH₄Cl and by KHCO₃ was very much decreased when the mitochondria were uncoupled by carbonyl cyanide *m*-chlorophenylhydrazone. It was consistently found that the stimulation by NH₄Cl was rather more sensitive to uncouplers than was that by KHCO₃.

The optimal conditions for glutaminase activity in intact mitochondria are further characterized in Table 1. A partial activation was obtained on addition of NH₄Cl plus KHCO₃ in the absence of a respiratory substrate. This activation was very much increased when succinate was also added to allow energization of the mitochondria. Glutaminase activity in this system was not activated by carbamoyl phosphate or by *N*-acetylglutamate over a 10 min time interval.

Table 1. Conditions for the maximum activity of glutaminase in liver mitochondria

Mitochondria were incubated at a concentration of 2.5–3.5 mg of protein/ml in a medium containing 120 mM-KCl, 20 mM-Tris/HCl, 15 mM-glutamine, 10 mM-potassium phosphate and 10 µg of rotenone/ml. Other additions were as indicated. The results shown are the means ± S.E.M. for triplicate determinations on the numbers of separate mitochondrial preparations shown in parentheses.

Additions	Rate of glutamate production (nmol/min per mg)
None	1.8 ± 0.7 (4)
Succinate (5 mM)	2.0 ± 0.5 (3)
NH ₄ Cl (5 mM) + KHCO ₃ (20 mM)	9.3 ± 2.3 (3)
Succinate + NH ₄ Cl + KHCO ₃	28.4 ± 3.4 (6)
Succinate + NH ₄ Cl + KHCO ₃ + amino-oxyacetate (0.5 mM)	26.2 ± 2.0 (3)
Succinate + NH ₄ Cl + KHCO ₃ + carbonyl cyanide <i>m</i> -chlorophenylhydrazone (0.5 µM)	5.9 ± 2.0 (5)
Succinate + NH ₄ Cl + KHCO ₃ + ornithine (20 mM)	26.1 ± 0.9 (3)
Carbamoyl phosphate (2 mM)	3.2 ± 1.3 (3)
Succinate + carbamoyl phosphate	3.7 ± 0.8 (3)
<i>N</i> -Acetylglutamate (1 mM)	2.3 ± 0.4 (3)

The maximum rate obtained in the presence of succinate, NH₄Cl plus KHCO₃ was not decreased by ornithine or by the transaminase inhibitor amino-oxyacetate. The lack of effect of this inhibitor indi-

Fig. 2. Dependence of mitochondrial glutaminase activity on the concentration of added NH₄Cl or of KHCO₃. Mitochondria (3 mg/ml) were incubated in a medium containing 120 mM-KCl, 20 mM-Tris/HCl, 5 mM-potassium succinate, 10 mM-potassium phosphate, 10 µg of rotenone/ml and 15 mM-glutamine. In (a), NH₄Cl was also present at the concentration shown. In (b), KHCO₃ was added at the concentration shown. The medium was not gassed. Incubations were carried out for 10 min. It should be noted that the KHCO₃ concentration shown represents only the initial HCO₃⁻ concentration before equilibration with the atmosphere has occurred.

cates that mitochondrial glutamine hydrolysis in liver is catalysed by glutaminase I rather than by the glutaminase II system involving glutamine-2-oxo acid transaminase. This conclusion is supported by the observation that the glutamine concentration required for a half-maximal rate of glutamine hydrolysis is 10–15 mM (Fig. 1), whereas the K_m of glutamine-2-oxo acid transaminase for glutamine is 2 mM (Cooper & Meister, 1977). The K_m for glutamine of rat liver glutaminase has been reported to be 28 mM (Huang & Knox, 1976).

Half-maximal stimulation of glutaminase activity was produced by the addition of 0.3 mM- NH_4Cl (Fig. 2a) or by approx. 3.5 mM- HCO_3^- (Fig. 2b). When glutaminase activity was stimulated by submaximal concentrations of NH_4Cl , the rate could be increased by the further addition of KHCO_3 ; similarly, the addition of low concentrations of KHCO_3 resulted in a stimulation that could be further increased by NH_4Cl . Glutaminase activity in intact mitochondria was found to be dependent also on the external phosphate concentration, as shown previously (Blackburn *et al.*, 1972). The phosphate dependence of liver glutamine-hydrolysing activity was originally observed by Carter & Greenstein (1947). A half-maximal rate was obtained at a phosphate concentration of 4 mM in the presence of saturating concentrations of NH_4Cl plus KHCO_3 (Fig. 3). The interpretation of the requirement for phosphate is unclear, but it may reflect in part the involvement of a phosphate-dependent glutaminase.

Nature of the energy requirements for glutaminase activity

It was shown in Table 1 that maximum rates of glutamine hydrolysis were obtained only in the presence of NH_4Cl or KHCO_3 plus a respiratory substrate. This finding could indicate a requirement for intramitochondrial ATP. Alternatively, it could indicate a requirement for a protonmotive force across the mitochondrial membrane to drive the accumulation of glutamine or phosphate or the energy-linked expulsion of glutamate, as is the case in kidney mitochondria (Crompton & Chappell, 1973). In principle, it is possible to discriminate between these possibilities by the use of oligomycin, which inhibits ATP synthesis without decreasing the protonmotive force.

It was found that, when mitochondria were incubated with NH_4Cl , KHCO_3 and succinate, the further addition of oligomycin inhibited glutaminase activity. However, the degree of inhibition was very variable, and consistent results were obtained only when the mitochondria were preincubated with oligomycin for at least 1 min before the addition of glutamine. In the experiment shown in Fig. 4 the intramitochondrial ATP concentration was varied by

Fig. 3. Dependence of mitochondrial glutaminase activity on the concentration of added phosphate in the presence of NH_4Cl and HCO_3^- .

The incubation medium contained 120 mM-KCl, 20 mM-Tris/HCl, 5 mM-potassium succinate, 10 μg of rotenone/ml, 15 mM-glutamine, 5 mM- NH_4Cl , 20 mM- KHCO_3 and 3.1 mg of mitochondrial protein/ml. Phosphate was added at various concentrations. Glutamate production was measured after 10 min.

preincubating mitochondria for times up to 6 min in the presence and absence of oligomycin. Glutamine was then added, and glutamate production was measured over the subsequent 10 min. Fig. 4 indicates that a correlation exists between the concentration of ATP in the mitochondria at the start of the incubation and the subsequent glutaminase activity. In contrast, no correlation was observed between glutaminase activity and the intramitochondrial ATP concentration at the end of the incubation period (results not shown). It was concluded that, in the presence of NH_4Cl plus KHCO_3 , the effective glutaminase activity is dependent on the concentration of intramitochondrial ATP present initially. Part of the requirement for phosphate (Fig. 3) therefore represents a requirement for ATP synthesis.

Glutaminase activity in disrupted mitochondria

It is not clear from the above results whether the transport of glutamine, glutamate or phosphate across the mitochondrial membrane may be rate-limiting for glutaminase activity in intact mitochondria. Information on this point may be obtained by studying glutaminase activity in disrupted mitochondria, where the permeability barrier is removed.

Fig. 4. Correlation between glutaminase activity and intramitochondrial ATP

Mitochondria (3mg/ml) were preincubated in a medium containing 120mM-KCl, 20mM-Tris/HCl, 10mM-potassium phosphate, 5mM-potassium succinate, 10 μ g of rotenone/ml, 20mM-KHCO₃ and 5mM-NH₄Cl at pH7.3 and 37°C for intervals between 0 and 5min. In some incubations, 10 μ g of oligomycin/ml was also present. After the preincubation period, 15mM-glutamine was added and a sample of the mitochondrial suspension was immediately deproteinized and subsequently assayed for ATP. Glutamate production was assayed after a further 10min. The results shown are those obtained from two separate mitochondrial preparations. Symbols: ○, preincubation with oligomycin; ●, preincubation without oligomycin.

of the mitochondria by sonication yielded very variable activities of glutaminase. Consistent results were obtained when the mitochondrial membrane was broken by repeated freezing and thawing of the mitochondria in a phosphate-containing medium, and this method was used for all subsequent experiments.

It was found that the dependence of glutaminase activity on phosphate in disrupted mitochondria was similar to that in intact mitochondria. In the presence of ATP, KHCO₃ and NH₄Cl at saturating concentrations, a half-maximal rate of hydrolysis of 15mM-glutamine was obtained at a phosphate concentration of approx. 5mM (results not shown).

Fig. 5 shows the characteristics of glutaminase activity in mitochondria that had been disrupted by repeated freezing and thawing. The activity of glutaminase in the absence of activators was very low, and the addition of ATP alone to the system was without effect (Fig. 5a). NH₄Cl (5mM) activated glutaminase to a smaller extent than in intact mitochondria, but this activation was much increased in the presence of ATP (Fig. 5a). HCO₃⁻ also activated the enzyme, and the HCO₃⁻ activation was also increased by ATP (Fig. 5b). In Fig. 5(c) it is shown that the activation produced by NH₄Cl and that by KHCO₃ in the absence of ATP were additive. Maximum rates were, however, obtained only in the presence of NH₄Cl plus KHCO₃ plus ATP. In the presence of ATP, the concentrations of NH₄Cl and of KHCO₃ required for half-maximal stimulation of glutaminase activity were similar to those shown in Fig. 3 for intact mitochondria. It may be noted from Fig. 5 that the maximum rate of glutaminase activity in the presence of activators is only slightly lower in disrupted mitochondria than in intact mitochondria. Further, the dependence of the fully activated system on glutamine concentration is similar in both intact and disrupted mitochondria. These results suggest that the transport of glutamine, glutamate and phosphate does not limit the activity of glutaminase in liver mitochondria.

The finding that maximum rates of glutaminase activity in disrupted mitochondria required the addition of NH₄Cl, KHCO₃ plus ATP suggested that glutaminase may in fact be activated by carbamoyl phosphate; disrupted mitochondria possess a high activity of the enzyme carbamoyl phosphate synthase (EC 2.7.2.5). In ten separate experiments with separate preparations of mitochondria, it was shown that the addition of 2mM-carbamoyl phosphate to disrupted mitochondria did not increase glutaminase activity. In disrupted mitochondria, maximum rates of carbamoyl phosphate synthesis are obtained only in the presence of added Mg²⁺ and *N*-acetylglutamate. Addition of these reagents was found to have no effect on glutaminase activity. Ornithine, added in excess, would remove any endogenous carbamoyl

Preliminary experiments showed that solubilization of the mitochondria with the detergent Triton X-100 completely inhibited glutaminase activity. Disruption

Fig. 5. Effect of KHCO_3 , NH_4Cl and ATP on glutaminase activity in mitochondria disrupted by repeated freezing and thawing

The mitochondrial suspension as prepared (65 mg of protein/ml) was diluted with an equal volume of 0.1 M-potassium phosphate, pH 7.4. The suspension was rapidly frozen and thawed three times in an acetone/solid CO_2 bath. Samples (3 mg) of the disrupted mitochondria were incubated in a medium containing (final concentrations): 120 mM-KCl, 20 mM-Tris/HCl, 10 mM-potassium phosphate and $10 \mu\text{g}$ of oligomycin/ml to inhibit ATP hydrolysis in this system. Other additions were 5 mM- NH_4Cl , 20 mM- KHCO_3 and 2 mM-ATP as indicated. (a): Δ , No additions; $\blacktriangle$, +ATP; $\circ$, + NH_4Cl ; $\bullet$, + NH_4Cl +ATP. (b): $\circ$, + KHCO_3 ; $\bullet$, + KHCO_3 +ATP. (c): $\circ$, + NH_4Cl + KHCO_3 ; $\bullet$, + NH_4Cl + KHCO_3 +ATP.

phosphate owing to the high activity of ornithine transcarbamoylase in disrupted mitochondria (McGivan *et al.*, 1976). However, the addition of 20 mM-ornithine did not affect the activity of the glutaminase in the presence of NH_4Cl , KHCO_3 and ATP (results not shown). Thus no evidence could be found to support the suggestion that carbamoyl phosphate activates the system.

Further experiments with disrupted mitochondria (results not shown) established that glutaminase was activated by 5 mM- NH_4Cl , but not by 5 mM-methylamine hydrochloride in the presence of ATP. When NH_4Cl was present, the activity of glutaminase could be increased by the further addition of ATP (Fig. 5), but not by 2 mM-ADP, -AMP, -IMP or -GTP. The activating effect of KHCO_3 could not be mimicked by the addition of 20 mM- KNO_3 , K_2SO_4 , KCNS , KI_3 or potassium formate, salts that may be regarded as analogues of HCO_3^- in certain respects.

The precise enzyme responsible for the hydrolysis of glutamine in liver mitochondria has not been identified in the present study. More than one enzyme may be involved. Contribution by glutamine transaminase can probably be excluded because of lack of inhibition by amino-oxyacetate. The contribution of P_i -independent glutaminase [which, in kidney, has been shown to be identical with glutamine transpeptidase (Curthoys & Kuhlenschmidt, 1975; Tate & Meister, 1975)] may be assessed by examining the effect of maleate on the mitochondrial system, since this enzyme is known to be activated by maleate (Katunuma *et al.*, 1966).

Mitochondria were disrupted by repeated freezing and thawing and were incubated in the presence of 15 mM-glutamine plus 10 mM-phosphate. The addition of 10 mM-maleate to this system gave an activation that was less than 5% of that obtained by the addition of NH_4Cl , KHCO_3 plus ATP under the conditions

described in the legend to Fig. 5. Further, the activated rate in the presence of these compounds was not affected by the further addition of maleate (10mM). From these results we concluded our mitochondrial preparations are not contaminated with tissue fragments possessing P_i -independent glutaminase activity.

Attempts were made to study the transport of glutamine into intact mitochondria. It was found that glutamine equilibrated very rapidly across the inner membrane and the time course of the equilibration could not be resolved by the conventional centrifugal-filtration techniques described in detail by McGivan *et al.* (1977). Glutamine equilibrated across the membrane at a range of added concentrations between 0 and 20mM; no accumulation was observed. The results did not permit the identification of any possible glutamine-transporting system. Whether or not such a transporting system exists in liver mitochondria, it is clear that the equilibration of glutamine across the membrane is very rapid, and it would seem that the rate of glutamine entry into the mitochondria would not be rate-limiting for glutaminase activity.

Discussion

The results obtained in the present investigation show that the regulation of glutaminase activity in mitochondria is a complex process. In the absence of activators, the activity of the enzyme is very low, even at high glutamine concentrations. Glutaminase activity is activated by NH_4Cl and also by HCO_3^- ions, but these activators exert their maximum effect only in the presence of ATP and phosphate. In intact mitochondria, ATP is provided by oxidative phosphorylation, and maximum activities of glutaminase are obtained only when the mitochondria are coupled. In disrupted mitochondria ATP must be added to achieve maximum rates of glutamine hydrolysis. In both intact (Fig. 1) and disrupted (Fig. 5) mitochondria the stimulations produced in the absence of ATP by NH_4Cl and $KHCO_3$ are additive. In the presence of ATP, NH_4Cl and HCO_3^- separately stimulate glutaminase to about 90% of its maximum activity. A comparison of the results shown in Figs. 1 and 5 indicates that the permeability properties of the mitochondrial membrane do not greatly influence glutaminase activity. It follows that the effects of bicarbonate, NH_4Cl and ATP are direct effects on the enzyme system rather than indirect effects on transmembrane pH gradients or mitochondrial metabolite concentrations. The nature of the interaction of these activators with glutaminase is unknown, and its elucidation will require data on the isolated enzyme. It is of interest that brain glutaminase is activated by ITP (Weil-Malherbe, 1969).

Certain differences are apparent between the enzyme systems responsible for the hydrolysis of glutamine in liver and kidney-cortex mitochondria. In both types of mitochondria, glutamine hydrolysis is an energy-dependent process. In kidney-mitochondria, glutamine hydrolysis requires the existence of a protonmotive force for the accumulation of phosphate and for the active extrusion of glutamate, which is a potent inhibitor of glutaminase (Kovacevic *et al.*, 1970; Crompton & Chappell, 1973). Glutamine is rapidly oxidized by kidney mitochondria and the glutamine concentration required for a maximal rate of oxidation is approx. 2mM (Hird & Marginson, 1968). In these mitochondria, glutamine is effectively accumulated via an electrogenic glutamine/glutamate antiport system (Crompton & Chappell, 1973). In contrast, the energy requirement for glutaminase activity in liver mitochondria reflects mainly a requirement for mitochondrial ATP. Glutamine is not accumulated in liver mitochondria. In contrast to kidney glutaminase, liver glutaminase is not inhibited by glutamate (Krebs, 1935). The concentration of glutamine required for half-maximal activation of glutamine hydrolysis in liver mitochondria is 10–15mM in the presence of NH_4Cl or HCO_3^- . The importance of these findings with regard to the control of glutamine hydrolysis in liver and kidney is not yet clear.

In isolated liver cells, glutamine metabolism is markedly stimulated by NH_4Cl (Joseph & McGivan, 1978). If the regulatory properties of glutaminase in isolated mitochondria reflect those in the intact cell the effects of NH_4Cl on cells can be explained by postulating that glutaminase is submaximally activated by HCO_3^- in the intact cell; the addition of NH_4Cl further activates the enzyme.

Glutamine metabolism in hepatocytes is stimulated by glucagon to a much greater extent than is the metabolism of other amino acids. It has been shown that the activation of glutamine metabolism is due to a stimulation of glutaminase (Joseph & McGivan, 1978). Injection of glucagon into rats has been shown to increase the rates of pyruvate carboxylation (Adam & Haynes, 1969) and citrulline synthesis from ornithine and NH_4Cl (Yamazaki & Graetz, 1977) in the subsequently isolated liver mitochondria. It is noteworthy that these processes, like glutamine hydrolysis, require ATP and HCO_3^- ions.

We thank Miss J. Ramsell for her technical assistance. This work was supported by a Project Grant from the Medical Research Council. S. K. J. gratefully acknowledges financial support from the Science Research Council.

References

- Adam, P. A. J. & Haynes, R. C. (1969) *J. Biol. Chem.* **244**, 6444–6450

- Bernt, E. & Bergmeyer, H. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 381–388, Academic Press, New York
- Blackburn, E. H., Hird, F. J. R. & Jones, I. K. (1972) *Arch. Biochem. Biophys.* **152**, 265–271
- Carter, C. E. & Greenstein, J. P. (1947) *J. Natl. Cancer Inst.* **7**, 433–445
- Chappell, J. B. & Hansford, R. G. (1972) in *Subcellular Components: Preparation and Fractionation* (Birnie, G. D., ed.), 2nd edn., pp. 77–91, Butterworth, London
- Charles, R. (1968) Ph.D. Thesis, University of Amsterdam
- Cooper, A. J. L. & Meister, A. (1977) *Crit. Rev. Biochem.* **4**, 281–303
- Crompton, M. & Chappell, J. B. (1973) *Biochem. J.* **132**, 35–46
- Curthoys, N. P. & Kuhlenschmidt, T. (1975) *J. Biol. Chem.* **250**, 2099–2105
- Gornall, H. G., Bardawill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751–756
- Hird, F. J. R. & Marginson, M. A. (1968) *Arch. Biochem. Biophys.* **127**, 718–724
- Huang, Y. Z. & Knox, W. E. (1976) *Enzyme* **21**, 408–426
- Ishikawa, E. (1975) *Adv. Enzyme Regul.* **14**, 117–136
- Joseph, S. K. & McGivan, J. D. (1978) *Biochim. Biophys. Acta*, **563**, 16–28
- Kalra, J. & Brosnan, J. T. (1973) *FEBS Lett.* **37**, 325–328
- Katunuma, N., Huzino, A. & Tomino, I. (1965) *Adv. Enzyme Regul.* **5**, 55–69
- Katunuma, N., Tomino, I. & Nishino, H. (1966) *Biochem. Biophys. Res. Commun.* **22**, 321–328
- Kovacevic, Z., McGivan, J. D. & Chappell, J. B. (1970) *Biochem. J.* **118**, 265–273
- Krebs, H. A. (1935) *Biochem. J.* **29**, 1951–1969
- Krebs, H. A., Lund, P. & Stubbs, M. (1976) in *Gluconeogenesis* (Hanson, R. W. & Mehlman, M., eds.), pp. 269–291, John Wiley and Sons, New York
- Lund, P. (1971) *Biochem. J.* **124**, 653–660
- Lund, P. & Watford, M. (1976) in *The Urea Cycle* (Grisolia, S., Baguena, R. & Mayor, R., eds.), pp. 479–488, John Wiley and Sons, New York
- McGivan, J. D., Bradford, N. M. & Mendes-Mourao, J. (1976) *Biochem. J.* **154**, 415–421
- McGivan, J. D., Bradford, N. M. & Beavis, A. D. (1977) *Biochem. J.* **162**, 147–156
- Stanley, P. E. & Williams, S. G. (1969) *Anal. Biochem.* **29**, 381–392
- Tate, S. S. & Meister, A. (1975) *J. Biol. Chem.* **250**, 4619–4627
- Weil-Malherbe, H. (1969) *J. Neurochem.* **16**, 855–864
- Yamazaki, R. K. & Graetz, G. S. (1977) *Arch. Biochem. Biophys.* **178**, 19–25