Biochem. J. (1977) **164**, 331–337
Printed in Great Britain

The Transport and Metabolism of Glutamine by Kidney-Cortex Mitochondria from Normal and Acidotic Rats

By JOHN T. BROSNAN and BEATRICE HALL
Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1C 5S7, Canada

(*Received* 5 *October* 1976)

1. The oxidation of glutamine by kidney-cortex mitochondria from normal and acidotic rats was not inhibited by avenaciolide, which did inhibit glutamate uptake and oxidation. The oxidation of glutamine by these mitochondria was always greater than that of glutamate. Direct measurements of the metabolism of [1-^{14}C]glutamine in the presence of glutamate, and of [1-^{14}C]glutamate in the presence of glutamine, demonstrated that the uptake and metabolism of external glutamate is insufficient to account for the observed rate of glutamine uptake and metabolism. Thus the postulated glutamine/glutamate antiport does not play a quantitatively important role in the metabolism of glutamine by renal mitochondria. 2. Rapid swelling of these mitochondria was observed in iso-osmotic solutions of L-glutamine and L-glutamyl-γ-monohydroxamate but not in D-glutamine or L-isoglutamine (1-amido-2-aminoglutaric acid). Thus a relatively specific glutamine uniport exists in these mitochondria. 3. The utilization of glutamine was increased about 3-fold in mitochondria from chronically acidotic rats. Thus mitochondrial adaptations play an important part in the renal response to metabolic acidosis.

Phosphate-dependent glutaminase (EC 3.5.1.2) has been shown to be located inside the inner membrane of mitochondria from pig kidney (Crompton *et al.*, 1973) and rat kidney (Kalra & Brosnan, 1974; Curthoys & Weiss, 1974). Thus the renal metabolism of glutamine by glutaminase necessitates the transport of this amino acid across the inner mitochondrial membrane. Pitts (1972) suggested that this transport process could be rate-limiting for the subsequent hydrolysis of glutamine and thus could exert a regulatory influence on renal ammonia production. The mechanism of glutamine transport into mitochondria has not yet been defined. Evidence for the existence of a glutamine/glutamate antiport in mitochondria from pig kidney has been presented by Crompton & Chappell (1973), but a glutamine uniport has also been proposed by Kovacevic *et al.* (1970). An understanding of the mechanism of glutamine transport into kidney mitochondria is important in delineating the pathway of glutamine metabolism in this tissue. A pathway using a glutamine uniport would be substantially different from one involving a glutamine/glutamate antiport. Knowledge of the transport mechanism is also essential for the design of suitable experiments for the measurement of the rate of transport. Clearly, different types of experiments would be needed depending on whether a glutamine/glutamate antiport or a glutamine uniport were involved. The present paper is therefore concerned with defining the mechanism of glutamine uptake by kidney-cortex mitochondria from normal and acidotic rats.

Materials and Methods

Biological materials

Male Sprague–Dawley rats (200–300g) were obtained from either the breeding colony maintained by the Memorial University Medical School or from Canadian Breeding Laboratories, La Prairie, Quebec, Canada. Control animals had free access to food and water. Chronic metabolic acidosis was induced by replacing the drinking water with a 1.5% solution of NH_4Cl for 7–10 days.

Mitochondria were prepared from kidney cortices as described by Kalra & Brosnan (1974). Mitochondria from both normal and acidotic rats were intact, as shown by high respiratory-control ratios, absolute latency of glutamate dehydrogenase (EC 1.4.1.3) and inability to oxidize externally added NADH.

Chemicals

All routine chemicals were of the highest grade commercially available and were purchased from either Sigma Chemical Co., St. Louis, MO, U.S.A. or from Boehringer Mannheim Corp., Montreal, Canada. L-Isoglutamine (1-amido-2-aminoglutaric acid) was obtained from K & K Laboratories, Plainview, NY, U.S.A.). Avenaciolide was a kind gift from Dr. W. B. Turner, Imperial Chemical

Industries Ltd., Macclesfield, Cheshire, U.K. DL-[1-^{14}C]Glutamate (1–5mCi/mmol) was purchased from New England Nuclear Corp., Montreal, Canada. All lots used were shown to contain 48–50% of the L isotope by the method given below for determining the radioactivity in the 1-position of L-glutamate. [1-^{14}C]Glutamine was prepared from DL-[1-^{14}C]glutamate by the following procedure. Glutamine synthetase (EC 6.3.1.2) was partially purified from sheep brain by steps I and II of the method of Rowe *et al.* (1970). Then 7ml of a medium containing 50mM-imidazole/HCl (pH7.2), 20mM-$MgCl_2$, 25mM-2-mercaptoethanol, 100mM-NH_4Cl and 10mM-ATP was incubated at 37°C for 2h with 0.2ml of DL-[1-^{14}C]glutamate (0.2mCi at 1–5mCi/mmol) and 0.5ml of the purified glutamine synthetase. At the end of the incubation, the entire incubation mixture was applied to a column (0.5cm × 9.0cm) of Dowex AG 1 (X8; formate form), and the glutamine was eluted with water (Adam & Simpson, 1974). After freeze-drying, the radioactive glutamine was dissolved in a small volume of water and was stored at −20°C. Since sheep brain glutamine synthetase reacts with both D- and L-glutamate it was necessary to determine the proportion of L-glutamine present. This was accomplished by incubating the glutamine with *Escherichia coli* glutaminase (Sigma, type V), which will hydrolyse only the L-glutamine. After incubation, the L-glutamate was separated from the D-glutamine by chromatography on a column (0.5cm × 4cm) of Dowex AG 1 (X8; formate form) (Adam & Simpson, 1974). In general, the synthesized [1-^{14}C]glutamine consisted of about 70% L-glutamine and 30% D-glutamine as determined by radioactivity measurement.

Experimental

Oxygen consumption by mitochondria was measured at 25°C by using a Clark-type electrode in a vessel of volume 1.8ml. The incubation medium (pH7.2) contained 0.14M-KCl, 5mM-Tris/HCl, 15mM-KH_2PO_4, 1mM-$MgCl_2$, 2mM-EDTA, 1mM-ADP and 1mg of bovine serum albumin (fat free)/ml. Incubations in the presence of labelled substrates were also carried out in the above medium. The final specific radioactivities of the labelled substrates were 20mCi/mol for glutamine and 200mCi/mol for glutamate. The mitochondria and media were placed in 25ml Erlenmeyer flasks, equipped for CO_2 collection, and were shaken at 25°C in a Dubnoff metabolic incubator at 100 strokes/min. The reaction was terminated and $^{14}CO_2$ collected and counted for radioactivity as described by Ambus *et al.* (1970). After CO_2 collection, the acidic medium was neutralized with KOH and portions were taken for measurement of 2-oxo[1-^{14}C]glutarate and of [1-^{14}C]-glutamate. $^{14}CO_2$ was released from the 1-position of 2-oxoglutarate by treatment with H_2O_2; 1.5ml of neutralized sample was incubated for 30min at 37°C with 15μl of H_2O_2 (30%, w/v) in a 25ml Erlenmeyer flask fitted with a rubber septum and a centre cell containing 0.2ml of NCS (Amersham-Searle Corp., Don Mills, Ontario, Canada). Then 0.3ml of 0.5M-H_2SO_4 was injected through the rubber septum, the shaking was continued and $^{14}CO_2$ collected and counted for radioactivity as described by Ambus *et al.* (1970). The sum of [1-^{14}C]glutamate plus 2-oxo[1-^{14}C]glutarate was measured by enzymically converting glutamate into 2-oxoglutarate and then releasing $^{14}CO_2$ from the 1-position of 2-oxoglutarate by treatment with H_2O_2. The glutamate is converted into 2-oxoglutarate by the method described by Lowry & Passonneau (1972) for the spectrophotometric determination of glutamate. To 1ml of sample in a 25ml Erlenmeyer flask fitted with a centre well containing NCS solution is added 1ml of a solution containing 50mM-Tris/HCl buffer (pH7.4), 1mM-NAD^+, 0.1mM-ADP, 1mM-EDTA and 10mM-H_2O_2. Then 0.2mg of ox liver glutamate dehydrogenase (EC 1.4.1.3) in 50% (v/v) glycerol solution was added and the mixture was incubated for 90min at 37°C. An excess of H_2O_2 [0.2ml of a 7% (w/v) solution] was then added and shaking continued for a further 1h, after which time 0.4ml of 0.5M-H_2SO_4 was added and the evolved $^{14}CO_2$ collected and counted for radioactivity as follows. The contents of the centre well were added to 15ml of a scintillation fluid made by dissolving 4g of Omnifluor (New England Nuclear Corp.) in 1 litre of toluene and were counted for radioactivity in a Beckman LS-23 scintillation counter. Corrections for quenching were made by the channels-ratio method.

Glutamate was measured spectrophotometrically by the procedure of Bernt & Bergmeyer (1974).

Mitochondrial swelling was measured at 25°C by following the change in A_{640} of a suspension of mitochondria by using a Beckman DB-G spectrophotometer and a Beckman 10in recorder. The media for these experiments contained 2mM-Hepes,* (pH7.2) and 1mM-EGTA and 0.25M-L-glutamine, D-glutamine, L-isoglutamine or L-glutamyl-γ-monohydroxamate. When swelling of mersalyl-treated mitochondria was studied, the mitochondrial pellet was resuspended in the homogenizing medium of Kalra & Brosnan (1974) containing 1mM-mersalyl {sodium *o*-[(hydroxymercuri-2-methoxypropyl) carbamoyl]phenoxyacetate} supplied by Sigma and was then shaken gently for 5min at 0°C. Unbound mersalyl was then removed by washing and the treated mitochondria were resuspended in homogenizing medium without mersalyl. Control mitochondria were treated identically except for the absence of mersalyl.

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

Results and Discussion

Experiments with the oxygen electrode

Our initial experiments were concerned with testing for the existence of a glutamine/glutamate antiport in renal-cortex mitochondria from the rat. Since the first step of glutamine metabolism in these mitochondria is its non-oxidative hydrolysis by glutaminase, it follows that the oxidation of glutamine is completely due to the oxidation of the glutamate so produced. If one glutamate molecule is obligatorily extruded from the mitochondria for each molecule of glutamine that enters (antiport hypothesis), it follows that the subsequent uptake of that glutamate is necessary to explain the rapid oxidation of glutamine by kidney mitochondria. Thus inhibition of glutamate uptake should decrease glutamine oxidation.

Avenaciolide has been shown to inhibit, in a competitive manner, the transport of glutamate into rat liver mitochondria (McGivan & Chappell, 1970). The effects of different concentrations of avenaciolide on the oxidation of glutamate and glutamine by kidney-cortex mitochondria from normal rats are demonstrated in Fig. 1. A short preincubation of the mitochondria with avenaciolide was necessary to obtain optimum effects. Low concentrations (10–25 μM) of avenaciolide caused a marked inhibition of glutamate oxidation. These concentrations are similar to, or less than, those shown by McGivan & Chappell (1970) to inhibit glutamate entry into rat liver mitochondria. When glutamine was the substrate, oxygen consumption was markedly increased by the addition of low concentrations of avenaciolide. The inhibitory effect of avenaciolide on glutamate oxidation and the stimulatory effect on glutamine oxidation were also evident in mitochondria from chronically acidotic rats (results not shown). At higher (100 μM) avenaciolide concentrations, the oxidation of both glutamate and glutamine was inhibited (Fig. 1), as was that of 2-oxoglutarate and of succinate (results not shown). This agrees with the observation by McGivan & Chappell (1970) that high concentrations of this substance inhibit respiration non-specifically. No evidence of uncoupling of oxidative phosphorylation was found at concentrations of avenaciolide up to 25 μM. Kovacevic (1975) has also shown that avenaciolide inhibits glutamate oxidation but not glutamine oxidation.

The antiport model for glutamine entry would also predict that the rates of oxidation of different concentrations of glutamate would be greater than that of similar concentrations of glutamine. This is so because, in the short time-interval that elapses before maximum rates of glutamine oxidation are observed, only a small fraction of the added glutamine is converted into glutamate. This is illustrated by experiments where the generation of extramitochondrial glutamate by mitochondria from normal and chronically acidotic rats was determined. After 5 min incubation, when maximum rates of glutamine oxidation have already been achieved, external glutamate concentrations of only 0.1 mM were generated from 2.5 mM-glutamine (Fig. 2). The antiport hypothesis would imply that the oxygen consumption observed with 2.5 mM-glutamine as substrate is completely due to the uptake and oxidation of this 0.1 mM-glutamate. Thus the rate of oxygen consumption with 0.1 mM-glutamate as substrate would be equivalent to that in the presence of 2.5 mM-glutamine. We examined the oxygen consumption of mitochondria from the kidney cortices

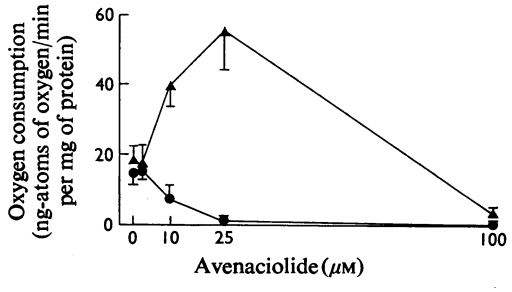

Fig. 1. *Oxidation of 2 mM-glutamine (▲) and 2 mM-glutamate (●) by rat kidney mitochondria in the presence of avenaciolide*
The mitochondria were incubated for 3 min with avenaciolide before the addition of substrate. Further details are given in the Materials and Methods section. The vertical bars represent one standard deviation ($n = 4$).

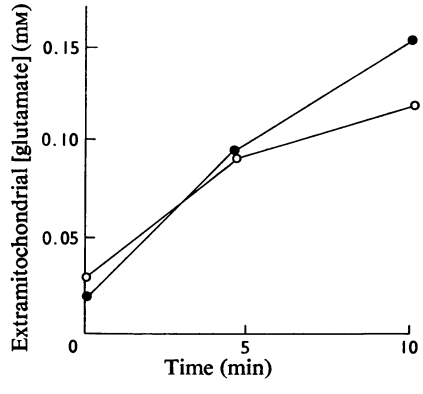

Fig. 2. *Production of extramitochondrial glutamate from 2.5 mM-glutamine by kidney mitochondria from normal (○) and chronically acidotic (●) rats*
Mitochondria (2.2 mg) were incubated in a final volume of 1.7 ml. Further details are given in the Materials and Methods section. Data are from a typical experiment.

of normal and acidotic rats in the presence of various concentrations of glutamine and glutamate (Fig. 3). At all substrate concentrations used, glutamine was oxidized more rapidly than was glutamate. This implies that glutamate oxidation by these mitochondria is limited by its rate of entry. The oxidation of glutamine was greatly increased in mitochondria from acidotic rats, whereas that of glutamate was unchanged. The oxidation of 2.5mM-glutamine was 4.5-fold greater than that of 0.1mM-glutamate in mitochondria from normal rats and was 8-fold greater in mitochondria from acidotic rats.

Experiments with labelled glutamine and glutamate

The experiments reported in Figs. 1–3 are not consistent with the antiport hypothesis. However, the rates of oxygen consumption in the presence of a substrate are not necessarily proportional to the rate of substrate uptake, since total oxygen consumption is determined both by the extent to which a substrate is metabolized as well as by the extent to which oxidation of endogenous substrates may be stimulated or decreased. Further, it is conceivable that in the presence of glutamine there could occur a stimulation of glutamate uptake such that the oxidation of external glutamate could now be sufficiently rapid to account for the oxidation of glutamine. Thus definitive results may be obtained only by experiments in which the rates of radioactive-substrate utilization are directly measured.

When [1-^{14}C]glutamine is metabolized by kidney mitochondria, it is first converted into [1-^{14}C]-glutamate by glutaminase, and this, in turn, is converted into 2-oxo[1-^{14}C]glutarate either by glutamate dehydrogenase or by aspartate aminotransferase (EC 2.6.1.1). $^{14}CO_2$ is next released from the 2-oxoglutarate dehydrogenase. Thus the extent of glutamine metabolism may be estimated from the production of labelled glutamate, 2-oxoglutarate and CO_2 from [1-^{14}C]glutamine. Similarly, glutamate metabolism may be estimated from the production of labelled 2-oxoglutarate and CO_2 from [1-^{14}C]glutamate. Therefore we measured the rate of utilization of 2.5mM-[1-^{14}C]glutamine in the presence of 0.1mM-glutamate and also the rate of utilization of 0.1mM-[1-^{14}C]glutamate in the presence of 2.5mM-glutamine. The concentration of glutamine used was chosen because it approximates to the concentration in kidney *in vivo* (Hems & Brosnan, 1971). The concentration of glutamate used was chosen because approximately this concentration of glutamate accumulates extramitochondrially during maximum rates of oxidation of 2.5mM-glutamine (Fig. 2). A possible source of error in some of these experiments arises from the fact that the production of unlabelled extramitochondrial glutamate from the unlabelled glutamine could markedly decrease the specific radioactivity of the 0.1mM-[1-^{14}C]glutamate during the course of the incubation. To minimize this source of error, the incubation volume was increased 3-fold from that used in the above experiments and the incubations were carried out for only 5min. The specific radioactivity of glutamate was always measured before and after the incubation. Decreases of the order of 30% were observed. This would introduce an underestimate of glutamate metabolism in the presence of glutamine of about 15% (assuming a constant rate of radioisotope dilution). No corrections were made for this error.

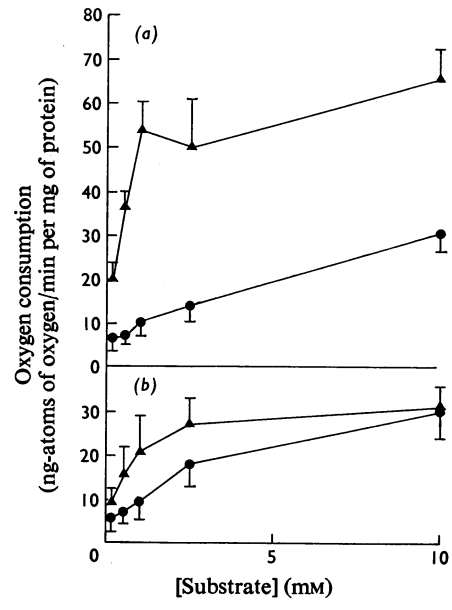

Fig. 3. *Oxygen consumption of kidney mitochondria from normal (b) and chronically acidotic (a) rats in the presence of various concentrations of glutamine and glutamate*
The vertical bars represent one standard deviation ($n = 4$). Further details are given in the Materials and Methods section. ▲, Glutamine; ●, glutamate.

The results of the experiments with labelled substrates are shown in Table 1. In mitochondria from normal rats the utilization of 2.5mM-glutamine in the presence of 0.1mM-glutamate amounted to 46.4nmol/5min per mg of protein, of which only 10nmol represented glutamate accumulation. The utilization of 0.1mM-glutamate in the presence of 2.5mM-glutamine amounted to only 7.8nmol/5min per mg of mitochondrial protein, and hence could in no way account for the rate of glutamine metabolism. The discrepancy was even greater in kidney mitochondria from acidotic rats, where the total glutamine utilization was increased to 113.7nmol/5min per mg of protein (of which only 17.5nmol was due to glutamate accumulation), whereas the oxidation of

Table 1. *Production of radioactive products from glutamine or glutamate by kidney-cortex mitochondria from normal and acidotic rats*

The experiments with glutamine represent the production of [1-^{14}C]glutamate, 2-oxo[1-^{14}C]glutarate and $^{14}CO_2$ from 2.5mM-[1-^{14}C]glutamine in the presence of 0.1mM-glutamate. The experiments with glutamate represent the production of 2-oxo[1-^{14}C]glutarate and $^{14}CO_2$ from 0.1mM-[1-^{14}C]glutamate in the presence of 2.5mM-glutamine. The data represent the means of four experiments ± S.E.M.

		Product (nmol/5min per mg of mitochondrial protein)		
Animal	Substrate	CO_2	2-Oxoglutarate	Glutamate
Normal	Glutamine	36.4±7.8	0.0±0.0	10.0±9.3
	Glutamate	7.3±2.3	0.5±0.05	—
Acidotic	Glutamine	93.7±12.7	2.5±1.3	17.5±0.0
	Glutamate	7.5±0.7	2.5±1.3	—

external glutamate under the same conditions amounted to only 7.5nmol/5min per mg of protein. Thus these experiments are totally inconsistent with a quantitatively important role for the proposed glutamine/glutamate antiport.

Mitochondrial swelling experiments

A uniport mechanism for the entry of glutamine into rat kidney-cortex mitochondria was originally proposed by Kovacevic *et al.* (1970) on the basis of the rapid swelling of these mitochondria in iso-osmotic glutamine solutions. In subsequent experiments using pig kidney mitochondria, Crompton & Chappell (1973) again observed rapid swelling in iso-osmotic glutamine, but swelling was greatly decreased when the glutamine concentration was lowered somewhat and sucrose was used to maintain iso-osmoticity. Since this uniport was most easily demonstrated with extremely high glutamine concentrations, Crompton & Chappell (1973) considered this mechanism to be of no physiological importance. We repeated these experiments of Crompton & Chappell (1973), but with rat kidney-cortex mitochondria. Under the conditions of Kovacevic *et al.* (1970), we observed rapid swelling when 0.25M-L-glutamine was used, but this swelling was almost eliminated when 0.187M-L-glutamine plus 0.063M-sucrose was used. Substitution of D-mannitol or KCl for the sucrose did not restore the rate of swelling. Hence these results with rat kidney-cortex mitochondria agree entirely with those obtained by Crompton & Chappell (1973) with pig kidney-cortex mitochondria and could be reasonably interpreted to exclude a physiological role for a uniport system. However, we also carried out other experiments on the effect of lowered concentrations of permeant solute (with ammonium phosphate as solute) on mitochondrial swelling measured spectrophotometrically. When mitochondria were swollen in 0.125M-sucrose/0.083M-ammonium phosphate the initial change in apparent absorbance was only about 10% of that observed in 0.166M-ammonium phosphate. Swelling in ammonium

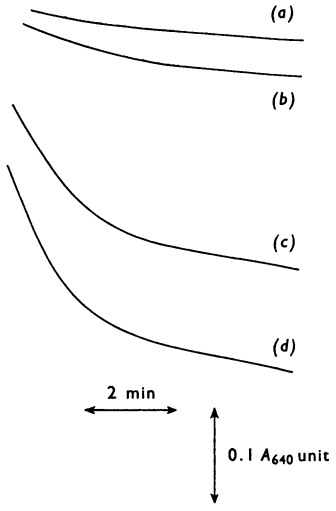

Fig. 4. *Swelling of rat kidney-cortex mitochondria in iso-osmotic solutions of glutamine or glutamine analogues*

The mitochondrial protein concentration was 0.25mg/ml. Further details are given in the Materials and Methods section. (*a*) L-Isoglutamine; (*b*) D-glutamine; (*c*) L-glutamyl-γ-monohydroxamate; (*d*) L-glutamine.

phosphate results from the activity of the well-established phosphate/hydroxyl antiport. Hence markedly less swelling of mitochondria when suspended in lower concentrations of permeant does not necessarily mean that the solute entry is brought about by an unphysiological mechanism. Therefore we examined further the swelling of mitochondria in solutions of L-glutamine.

The specificity of mitochondrial swelling in glutamine was examined (Fig. 4). Mitochondria did not swell appreciably in solutions of D-glutamine or of L-isoglutamine, but did swell in L-glutamine and in

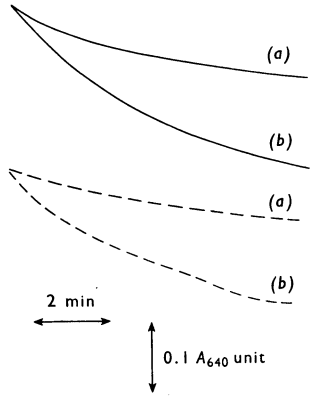

Fig. 5. *Effect of mersalyl treatment on the swelling of kidney mitochondria in solutions of glutamine and of glutamyl-γ-monohydroxamate*

The mitochondrial protein concentration was 0.25mg/ml. Further details are given in the Materials and Methods section. (*a*) Mersalyl-treated and (*b*) untreated mitochondria. ——, Glutamine; ----, glutamyl-γ-monohydroxamate.

solutions of L-glutamyl-γ-monohydroxamate, an analogue of glutamine. The effect of mersalyl on mitochondrial swelling was also examined (Fig. 5). The swelling of mitochondria from rat kidney cortex in L-glutamine or in L-glutamyl-γ-monohydroxamate was markedly inhibited in mersalyl-treated mitochondria. That such inhibition was due to an impairment in solute permeation rather than to decreased water uptake was demonstrated in experiments which showed that the extremely rapid swelling of mitochondria in iso-osmotic ammonium acetate was equally manifest in mersalyl-treated mitochondria.

Nature of the glutamine transporter

A uniport mechanism for glutamine transport into pig kidney mitochondria was rejected by Crompton & Chappell (1973) on the basis of the unsatisfactory swelling behaviour of these mitochondria. They proposed a glutamine/glutamate antiport and demonstrated, in accordance with such a mechanism, that the sum of [glutamine] plus [glutamate] in the matrix was constant despite variations in the relative amounts of the amino acids. Since aspartate was a major end product of glutamine metabolism by pig kidney-cortex mitochondria, Crompton & Chappell (1973) proposed that the re-uptake of glutamate was coupled to the extrusion of aspartate (glutamate/aspartate antiport). The conversion of glutamate into aspartate via the reactions described by Haslam & Krebs (1963) would then account for the observed oxygen consumption.

There are already several indications that such a scheme could not account for the uptake of glutamine by rat kidney mitochondria. Kovacevic *et al.* (1970) demonstrated that very high concentrations of glutamate (35mM) accumulate in the matrix of rat kidney mitochondria as a result of glutaminase action. Further, inhibition by amino-oxyacetate of the aspartate aminotransferase in rat kidney-cortex mitochondria completely abolishes aspartate production from glutamine, but is without effect on the rate of glutamine oxidation (Kovacevic, 1971). The experiments described in Figs. 1–3 and in Table 1 are completely inconsistent with any major role for the antiport mechanism in the oxidation of physiological concentrations of glutamine. In particular, the data in Table 1 directly demonstrate that the uptake and metabolism of external glutamate is insufficient to account for the simultaneous uptake and metabolism of glutamine. That the respiration of rat kidney mitochondria oxidizing glutamate is insufficient to account for that found with glutamine as substrate was recognized by Crompton & Chappell (1973). However, they believed that rat kidney mitochondria contained two glutaminases (phosphate-dependent glutaminase and phosphate-independent glutaminase) and suggested that the glutamine/glutamate antiport permitted access to the phosphate-dependent glutaminase, whereas access to the phosphate-independent glutaminase was via a different glutamine-transporting system. Thus, in view of the high phosphate-independent glutaminase activity in their mitochondrial preparations, it was possible that a glutamine/glutamate antiport could be functional even though the rates of glutamate oxidation were low. However, it is now known that phosphate-independent glutaminase is not a mitochondrial enzyme but is associated with the proximal-tubular brush border (Kalra & Brosnan, 1974). Further, it appears that this enzyme is not a true glutaminase but that the hydrolysis of glutamine is carried out by a side reaction of γ-glutamyltransferase (EC 2.3.2.2) (Tate & Meister, 1974; Curthoys & Kuhlenschmidt, 1975). Thus kidney mitochondria contain only one glutaminase, phosphate-dependent glutaminase, and therefore the low rate of utilization of external glutamate precludes a role for the proposed glutamine/glutamate antiport.

Our studies on mitochondrial swelling are consistent with the existence of a glutamine uniport. Since swelling does not occur in solutions of D-glutamine or of L-isoglutamine, the uniporter exhibits considerable specificity. The swelling in L-glutamine is a result of the uniport entry of L-glutamine. Some of the glutamine will be hydrolysed to glutamate by glutaminase, but this will also contribute to the matrix osmotic pressure. Comparable swelling was also observed in solutions of L-glutamyl-γ-monohydroxamate, a close analogue of glutamine.

Since phosphate-dependent glutaminase is only 0.5% as active towards this analogue as it is towards glutamine, it is evident that the mitochondrial swelling in solutions of L-glutamyl-γ-monohydroxamate is entirely due to the entry of this molecule and not at all to its metabolism. Additionally, this experiment casts doubt on the proposal by Curthoys & Weiss (1974) that phosphate-dependent glutaminase is a component of the glutamine-transporting system.

The inhibition by mersalyl of swelling in solutions of glutamine or of L-glutamyl-γ-monohydroxamate implies that the operation of the glutamine uniporter requires the presence of functional thiol groups. The uptake of lower concentrations of labelled glutamine by kidney mitochondria from rat and dog has also been shown to be inhibited by mersalyl (Adam & Simpson, 1974; Simpson & Adam, 1975; Goldstein, 1975). Thus the uniport demonstrable at high glutamine concentrations displays the same sensitivity to mersalyl as does the uptake mechanism operative at physiological glutamine concentrations.

The subcellular pathway of glutamine metabolism in cells is dependent on the nature of the mitochondrial glutamine transporter. Pitts *et al.* (1965) have shown, using glutamine labelled with ^{15}N in either the amino or amide positions, that the amino group of glutamine is an important source of urinary ammonia. Thus, if the antiport mechanism for glutamine transport were operative, the re-uptake of glutamate by mitochondria would be necessary for deamination by glutamate dehydrogenase to occur. This re-uptake has been suggested as a control site by Roobol & Alleyne (1974). However, our data do not support this proposal, and the simpler pathway whereby glutamate is produced in the matrix by glutaminase and is immediately deaminated by glutamate dehydrogenase probably occurs.

This work was supported by a grant from the Medical Research Council of Canada (MA-4643).

References

Adam, W. & Simpson, D. P. (1974) *J. Clin. Invest.* **54**, 165–174

Ambus, T., Dryden, E. E. & Manery, J. F. (1970) *Int. J. Biochem.* **1**, 553–556

Bernt, E. & Bergmeyer, H. U. (1974) in *Methods of Enzymatic Analysis*, 2nd edn. (Bergmeyer, H. U., ed.), pp. 1704–1708, Academic Press, New York

Crompton, M. & Chappell, J. B. (1973) *Biochem. J.* **132**, 35–46

Crompton, M., McGivan, J. D. & Chappell, J. B. (1973) *Biochem. J.* **132**, 27–34

Curthoys, N. P. & Kuhlenschmidt, T. (1975) *J. Biol. Chem.* **250**, 2099–2105

Curthoys, N. P. & Weiss, R. F. (1974) *J. Biol. Chem.* **249**, 3255–3260

Goldstein, L. (1975) *Am. J. Physiol.* **229**, 1027–1033

Haslam, R. J. & Krebs, H. A. (1963) *Biochem. J.* **88**, 566–578

Hems, D. A. & Brosnan, J. T. (1971) *Biochem. J.* **123**, 391–397

Kalra, J. & Brosnan, J. T. (1974) *J. Biol. Chem.* **249**, 3255–3260

Kovacevic, Z. (1971) *Biochem. J.* **125**, 757–763

Kovacevic, Z. (1975) *Biochim. Biophys. Acta* **396**, 325–334

Kovacevic, Z., McGivan, J. D. & Chappell, J. B. (1970) *Biochem. J.* **118**, 265–274

Lowry, O. H. & Passonneau, J. V. (1972) *A Flexible System of Enzymatic Analysis*, pp. 184–186, Academic Press, New York

McGivan, J. D. & Chappell, J. B. (1970) *Biochem. J.* **123**, 391–397

Pitts, R. F. (1972) *Kidney Int.* **1**, 297–305

Pitts, R. F., Pilkington, L. A. & de Haas, J. C. M. (1965) *J. Clin. Invest.* **44**, 731–745

Roobol, A. & Alleyne, G. A. O. (1974) *Biochim. Biophys. Acta* **362**, 83–91

Rowe, W. B., Rozio, R. A., Wellner, V. P. & Meister, A. (1970) *Methods Enzymol.* **17**, 900–910

Simpson, D. P. & Adam, W. (1975) *J. Biol. Chem.* **250**, 8148–8158

Tate, S. & Meister, A. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3329–3333