Avian Mitochondrial Glutamine Metabolism*

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Intact avian liver mitochondria were shown to synthesize glutamine from glutamate in the absence of exogenous ATP and ammonia. With L-[U-14C]glutamate as the substrate, there was an approximate 1:1 stoichiometry between glutamate deaminated (as measured by the release of 14CO2 due to α-keto-14C-glutarate oxidation) and glutamate amidated. With L-[15N]glutamate as the substrate, the isolated glutamine was shown by low and high resolution mass spectrometry of its phenylisothiocyanate derivative to contain 15N in both the α-amino and amide groups. Thus, for each mole of glutamate taken up, approximately 0.5 mol is deaminated and the other 0.5 mol serves as a substrate for glutamine synthetase previously localized in these mitochondria (Vorhaben, J. E., and Campbell, J. W. (1972) J. Biol. Chem. 247, 2763).

The permeability of L-glutamine to intact avian liver mitochondria was studied by a rapid centrifugation technique. Efflux as well as influx of L-glutamine were both rapid and appeared to occur by a passive, energy-independent process. These results indicate that the mitochondrial glutamine synthetase present in uricotelic species represents the primary ammonia detoxication reaction in that ammonia released intramitochondrially during amino acid catabolism is converted to glutamine for efflux to the cytosol where it may serve as a substrate for purine (uric acid) biosynthesis.

The detoxication of ammonia released during hepatic amino acid catabolism by its eventual conversion to uric acid for excretion by uricoles is an old and widely accepted concept. Although the pathway for purine synthesis de novo in these species has been known for many years, the nature and site of the primary ammonia detoxication step remained uninvestigated. Studies with the nonparticulate fraction of pigeon liver served to establish the precursors of the purine ring nitrogen. These studies also served to localize purine biosynthesis in the cytosol of avian liver cells (2). With the non-particulate fraction, the incorporation of ammonium nitrogen into the purine ring is minimal compared with the incorporation of the amide nitrogen of glutamine and the α-amino nitrogens of aspartic acid and glycine. Since birds, like mammals, presumably catabolize amino acids in liver tissue by the combined reactions of the cytosolic transaminases and mitochondrial glutamate dehydrogenase, ammonia generated intramitochondrially must thus ultimately be converted to a precursor of the purine ring for efflux to the cytosol. We have previously shown that glutamine synthetase is localized almost exclusively in chicken liver mitochondria and suggested that this enzyme represents the primary ammonia detoxication reaction (3). Glutamine synthetase in uricotelic species would then be equivalent to carbamyl phosphate synthetase I in uricotelic species. In direct support of this hypothesis, we show here that ammonia liberated intramitochondrially from glutamate is incorporated into the amide group of glutamine and that the glutamine synthesized readily effluxes the mitochondria.

EXPERIMENTAL PROCEDURES

Preparation of Mitochondria—Mitochondria were isolated from the livers of either Red Comb hybrid or White Leghorn chickens as described by Schneider (4). The pellet sedimenting at 6800 × g was taken as the mitochondrial fraction and was washed three times in cold 0.25 M sucrose. Respiratory control ratios (5) were measured with a Clark-type electrode. Values ranging from 2.4 to 3.5 were found with L-glutamate as the substrate. Mitochondrial protein was determined by the method of Lowry et al. (6) using bovine serum albumin as the standard.

Incubation with L-[U-14C]Glutamate—Mitochondria were incubated as described in the legend of Fig. 1. Duplicate incubations were carried out in 10-ml Erlenmeyer flasks capped with rubber septa into which polypropylene cups containing 0.2 ml of 1 M methanolic Hyamine hydroxide (p-(diisobutylcresoxyethoxyethylidimethylbenzyl ammonium hydroxide) were inserted to trap 14CO2. At the times indicated in Fig. 1, the cups were removed and placed directly into scintillation vials containing 10 ml of Bray’s solution (7) for counting by liquid scintillation spectrometry. To isolate [14C]glutamine, the reaction mixtures were centrifuged at 6800 × g for 10 min. The supernatant fluid was decanted and frozen at −20 °C. Unreacted substrate and [14C]glutamate in the thawed supernatant fluid were separated by paper electrophoresis and counted as previously described (8), except the separating buffer was 0.05 M ammonium formate, pH 7 to 7.2.

Incubation with L-[15N]Glutamate—Mitochondria (25.4 mg of protein) were incubated in 5.0 ml of the medium described in Fig. 1 except 22.5 mM L-[15N]glutamate (potassium salt, pH 7.4; 96.5% enriched in 15N) was substituted for [14C]glutamate. Following an incubation period of 60 min at 37 °C, the mitochondria were sedimented at 6900 × g for 10 min and were washed with cold 0.25 M sucrose. The supernatant fluid and washings were combined and the volume

*This work was supported by Grant GB-38138 from the National Science Foundation and Grant AI-05006 from the National Institute of Allergy and Infectious Diseases. A preliminary report of some of the data has been made (1).
Glutamine synthesis was also determined following sedimentations of this experiment were such that the exogenously glutamate under isosmotic conditions and the $^{14}CO_2$ released during metabolism was trapped and counted to estimate the oxidation of cu-ketoacid to glutamate and its quenching by tissue metabolites.

The osmolarity of all mitochondrial incubation mixtures was checked for isosmotic conditions. The osmolarity of the mitochondria was investigated by the rapid centrifugation method described in the Experimental Procedures. Following incubation for the times indicated, the mitochondria were sedimcnted and the $[^14]C$-glutamine synthesized was isolated from the supernatant fluid and counted, also as described in the text.

was reduced in a rotary evaporator. Paper electrophoresis was again utilized to separate glutamine and unreacted glutamate. Bands corresponding to these two amino acids were located by comparison with a control strip reacted with ninhydrin. Glutamine and glutamate were eluted from the cut strips with water, taken to dryness and their PTH-derivatives prepared according to the procedure of Edman and Begg (5). Purification of the PTH-derivatives was achieved by paper electrophoresis and repeated thin layer chromatography. Following electrophoresis as described above, the bands corresponding to the PTH-derivatives were detected by ultraviolet light quenching and were cut out and eluted with ethyl acetate followed by acetone. The eluates were reduced in volume and chromatographed on Analtech Silica Gel G thin layer plates containing a fluorescent indicator but gave no visible bands from the latter. Paper electrophoresis in the subsequent mass spectral analysis. The developing solvent was chloroform/methanol (90/10, by volume). The PTH-derivatives of glutamine and glutamic acid were again detected by ultraviolet quenching. The areas were scraped from the plates and extracted with ethyl acetate and then acetone, and the extracts were taken to dryness for analysis by low and high resolution spectrometry. That this separation protocol completely separated glutamine from glutamic acid present in the original reaction mixture was shown by using a mock reaction mixture containing 4 mM $L$-glutamine and 4 mM $L-[^14]C$glutamic acid (0.5 $\mu$Ci of total $[^14]C$). Isolation of glutamine and preparation and purification of its PTH-derivative from this reaction mixture were as described above. No radioactivity was detected in the isolated PTH-derivative of glutamine, showing the absence of any glutamic acid contamination.

Synthesis of $L-[^{15}N]$Glutamine Derivatives—$L$-Glutamine derivatives labeled with $^{15}N$ in the $\alpha$-amino function, in the amide function, and in both functions were synthesized from a combination of $[^15]N$ ammonium chloride and $[^15]N$glutamic acid, their unlabeled counterparts, and a commercial preparation of glutamine synthetase. Reaction mixtures contained: 43.5 mM Tris-chloride buffer, pH 7.4; 17 mM magnesium chloride; 17 mM ATP; 22 mM $\beta$-mercaptoethanol; 87 mM ammonium chloride; 87 mM potassium $L$-glutamate; pH 7.4; and 1.1 units (1 unit = 1 amol glutamine formed/15 min at 37$^\circ$) glutamine synthetase in a volume of 15 ml. This was incubated for 2 hours at 37$^\circ$ and the reaction was terminated by the addition of 2.5 ml of 1 M perchloric acid. The reaction mixture was centrifuged at 20,000 x $g$ for 10 min and the supernatant fluid was collected and neutralized with potassium hydroxide. The potassium perchlorate was removed by centrifuging and the supernatant fluid was reduced in volume. Isolation of glutamine from these samples and the preparation and purification of the PTH-derivatives were as described above.

Mass Spectrometry—Low resolution mass spectra were obtained with an LKB 9000 mass spectrometer utilizing the direct introduction probe. Minor impurities, probably extracted from the thin layer plates, were removed by partial distillation on the probe. Operating conditions were: ionizing voltage, 70 e.v.; accelerating voltage, 2.4 kv; and anode current, 100 $\mu$A. The ion source temperature was 270$^\circ$ and the probe temperature varied between 50 and 120$^\circ$. High resolution spectra were obtained with a DuPont CEC 21-110 mass spectrometer having a direct introduction probe. Operating conditions were: ionizing voltage, 70 e.v.; accelerating voltage, 8.4 kv; and anode current, 100 $\mu$A. Spectra were recorded on Q2 photoplates and line distances and intensities were measured with a Gaertner microdensitometer. Data were reduced to accurate masses, ±3 millimass units (mmu), with a computer (7).

Low resolution spectra of the unlabeled PTH-derivative of glutamine and the PTH-derivative of glutamic acid were corroborated from the literature (11).

Permeability Studies with $L-[^14]C$Glutamine—Permeability of avian liver mitochondria was investigated by the rapid centrifugation method using an Eppendorf 3200 microcentrifuge. Uptake studies were initiated by the addition of mitochondria to an isosmotic buffered medium containing $L-[^14]C$glutamine in 1.5-ml Eppendorf tubes. The reaction mixture components are described in Table II. Following incubation for the times indicated in Table II and Fig. 4, the mitochondria were rapidly sedimented for 2 min at 8000 x $g$ (maximum centrifugal force attained in less than 10 s). The supernatant fluid was quickly decanted and the surface of the pellet washed with cold 0.15 M sucrose. Excess fluid adhering to the sides of the tube was carefully removed by blotting. The mitochondrial pellets were solubilized in 0.3 to 0.5 ml of Amersham NCS solubilizer and counted in 10 ml of a toluene-Permablend (Packard Instruments) scintillation fluid. For efflux studies, mitochondria were first preloaded with $[^14]C$glutamine as described in Fig. 4. After recovery of the loaded mitochondria by centrifuging, they were resuspended in a glutamine-free medium for the times indicated in Fig. 4 and then centrifuged for 2 min at 8000 x $g$. The mitochondrial pellets were washed, solubilized, and counted as above.

The isomallarity of all mitochondrial incubation mixtures was checked with an Advanced Instruments spectrometer. Reagents—$L$-Glutamine, rotenone, ATP, and sheep brain glutamine synthetase were obtained from Sigma Chemical Co.; $L$-glutamic acid from Fisher Chemicals; phenylisothiocyanate from Eastman Organic Chemicals; $[^15]N$ ammonium chloride (99.5% enriched in $[^15]N$) from the Cambridge Isotope Laboratories and $[^15]N$glutamic acid (99.5% enriched in $[^15]N$) from Merck, Sharp and Dohme of Canada. All $[^14]C$ compounds and $H_2O$ were products of New England Nuclear. De-carboxylation of the $L-[^14]C$glutamic acid with glutamate decarboxylase yielded 20.8% of the total radioactivity in the carboxyl carbon. $[^14]C$Glutamine was routinely passed through a DEAE-cellulose column in the bicarbonate form to remove contaminating glutamic acid. Other chemicals were of reagent grade quality.

RESULTS

Mitochondrial Synthesis of $[^14]C$Glutamine—Support for the hypothesis that intramitochondrial glutamine synthesis represents the primary mechanism for detoxication of ammonia released via glutamate dehydrogenase in avian liver was initially obtained in studies on the mitochondrial synthesis of $[^14]C$glutamine from $[^14]C$ glutamate in the absence of exogenous ammonia and ATP. For the results shown in Fig. 1, washed avian liver mitochondria were incubated with $[^14]C$glutamate under isosmotic conditions and the $[^14]CO_2$ released was trapped and counted to estimate the oxidation of $\alpha$-keto$[^14]C$glutarate formed by deamination of the substrate. $[^14]C$-Glutamine synthesis was also determined following sedimentation of the mitochondria from the reaction mixture. The conditions of this experiment were such that the exogenously

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1 The abbreviation used is: PTH, phenylthiohydantoin.
added glutamate substrate provided all of the substrates for the intramitochondrial glutamine synthetase: ammonia, formed by the action of glutamate dehydrogenase; ATP, generated via the oxidation of α-ketoglutarate; and glutamate itself. As shown in Fig. 1, there is an apparent "burst" of glutamine synthesis during the first 10 min. Synthesis then becomes linear and remains so up to 60 min. There is an initial lag in α-ketoglutarate oxidation, as measured by CO₂ release, but this then also becomes linear. The observed initial "burst" in glutamine formation could possibly be due to endogenous ATP and ammonia. Neither of these were measured but are presumably present since they are required substrates for glutamine synthesis. As glutamate becomes available, it could be used preferentially for glutamine synthesis to deplete the mitochondria of ammonia. Glutamate oxidation then begins, as indicated by CO₂ production. Based on this interpretation, the results approach a reasonable 1:1 stoichiometry between glutamate deaminated (the release of 0.53 μmol of CO₂ is equivalent to the oxidation of 0.13 μmol of α-ketoglutarate) and glutamate amidated by the mitochondrial glutamine synthetase (0.18 μmol). α-Keto[¹³C]glutarate was not detected in this experiment, due, presumably, to its rapid oxidation. Since influx and efflux of α-ketoglutarate occurs with mammalian liver mitochondria (12, 13), it seems unlikely that the absence of α-keto[¹³C]glutarate in the supernatant fluid was due to its limited permeability. In other experiments in which a higher mitochondrial protein concentration was used (∼10 mg/ml), evidence for the presence of α-ketoglutarate was obtained. A labeled compound was present in the supernatant fluid which migrated with α-ketoglutarate during electrophoresis and fluoresced when reacted with o-phenylenediamine. Controls lacking glutamate did not contain this compound.

Compared with mammalian liver mitochondria, the O₂ uptake values for avian liver mitochondria with L-glutamate as the substrate were consistently low. With ADP as the phosphoryl acceptor, these values ranged from 10 to 16 nmol of O₂/min/mg of protein and are thus somewhat less than one-half the rates reported for mammalian mitochondria under similar conditions (30 to 40 nmol of O₂/min/mg of protein; Ref. 14). These O₂ uptake values are thus also consistent with the interpretation that only approximately one-half the glutamate taken up by these mitochondria is oxidatively deaminated.

**Mitochondrial Synthesis of [¹⁵N]Glutamine**—According to the hypothesis, glutamine synthesized by avian mitochondria provided with [¹⁵N]glutamate should be ¹⁵N-labeled in both the amino and amide functions, the amide-¹⁵N arising from [¹⁵N]ammonia released by glutamate dehydrogenase and the amino-¹⁵N from a second [¹⁵N]glutamate serving as substrate for glutamine synthetase. Our original intent was to determine the distribution of ¹⁵N in biosynthetic glutamine directly by low resolution mass spectrometry. However, both underivatized glutamine and glutamic acid gave yields of molecular ions of less than 10% of the base peak. An additional problem was the loss of the amide function in the glutamine spectrum. These problems were overcome by derivatizing the two amino acids. The PTH-derivatives proved quite satisfactory for analysis because of their high yield of molecular ion, retention of the amide function of the glutamine derivative and their ease of synthesis. In using the PTH-derivatives, it is important that the isolated glutamine be free of contaminating glutamate since low resolution spectrometry would not distinguish the small mass difference of 13 mmu between the molecular ions of [¹⁴N]glutamic acid (265.0539) and [amide, α-amino-¹⁴N]glutamine (265.0669). As described under "Experimental Procedures," contamination of the isolated PTH-derivative of glutamine was checked for by starting with a mixture of unlabeled glutamine and [¹³C]glutamate and determining ¹³C in the final product: the absence of ¹³C indicated the isolation procedure gave complete separation of the two amino acids.

**PTH-derivatives** of glutamate containing ¹⁵N in the amino function, the amide function, and in both functions were synthesized for use as standards in the mass spectrometric analyses. The structure of the molecular ion and major fragments of unlabeled PTH-derivatives of glutamine are shown in Fig. 2. Loss of ammonia produces a fragment of mass 246 and cleavage of the bond between the β and γ carbons yields an acetamide molecule of mass 59 and another fragment of mass 204. Both of these processes require a nonspecific rearrangement of hydrogen atoms. Bond cleavage between the β and γ carbons is of particular significance since this allows the amino and amide nitrogen to be distinguished. The fragment of mass 340 arises from PTH ring cleavage and the mass 77 fragment is due to the phenyl ring. These latter ions are common to the mass spectra of most PTH-derivatives (11). The low resolution mass spectrum of the unlabeled PTH-derivative of glutamine (not shown) exhibited peaks corresponding to the various fragments depicted in Fig. 2 and was essentially the same as reported by Hagenmaier et al. (11).

Low resolution mass spectra of each of the [¹⁵N]-labeled glutamine derivatives exhibited the predicted mass shifts. The PTH-derivative of [α-amino-¹⁵N]glutamate gave peaks at m/e values of 264, 247, and 205 representing shifts of 1 atomic mass unit (amu) and an unchanged peak at 59. The PTH-derivative of [amide-¹⁵N]glutamine gave peaks at 204 and 60, the expected shifts of 1 amu and unchanged peaks at 204 and 246. The mass spectrum of the PTH-derivative of [α-amino, amide-¹⁵N]glutamine is shown in Fig. 3. The predicted shifts in mass were again readily observed: at 204, a shift of 2 amu in the molecular ion, and at 247, 205, and 59, shifts of 1 amu. Also shown in Fig. 3 is the mass spectrum of the PTH-derivative of glutamine isolated from the mitochondrial incubation mixture. This spectrum is essentially identical with that of the doubly labeled standard and establishes the origin of the amide group synthesized by mitochondria from the amino nitrogen of glutamate.

For comparative purposes, the low resolution spectrum of the PTH-derivative of [¹⁵N]glutamic acid is also shown in Fig. 3. This spectrum is quite similar but not identical with that of doubly labeled PTH-derivative of glutamine. Critical quantitative differences occur at m/e values of 60, the amide moiety appearing in the glutamine spectrum, and at
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During oxidative deamination of glutamate is converted to the amide nitrogen of glutamine by glutamine synthetase. The experiments, especially the critical acetamide moiety, also showed a good correlation.

Confirmation of the low resolution data was obtained by accurate mass measurements (± 0.1 mmu) with a high resolution mass spectrometer. These results are shown in Table I. The mass of the molecular ion of the PTH-derivative of [a-amino, amide-15N]glutamine (PTH-GLN N-15 AMINO, N-15 AMIDE) of PTH-derivative of glutamine isolated from mitochondrial (PTH-GLN FROM MITO.) incubation with [15N]glutamate, and of [15N]glutamic acid (PTH-GLU N-15 AMINO). See text for discussion.

219, a fragment observed in the PTH-derivative of glutamic acid spectrum. The latter arises as a result of the loss of a formic acid moiety (11).

The efflux of L-glutamine from preloaded mitochondria is shown in Fig. 4. Under the conditions used for preloading (100 mM glutamine), the internal to external ratio was again greater than 1. As can be seen in this figure, at the shortest measured interval of “1 min”, glutamine had essentially equilibrated across the membrane (a 2-min centrifugation was required to sediment the mitochondria). An analysis of the radioactive products appearing in the supernatant fluid showed 91% of the 14C in glutamine and 8% in glutamate. The formation of glutamate is presumably due to some metabolism of glutamine in addition to nonenzymatic hydrolysis since preliminary observations indicate a low but detectable “glutaminase” activity in these mitochondria. The results of these permeability studies thus indicate that glutamine readily traverses the membrane of avian liver mitochondria in either direction by a passive, energy-independent process.

DISCUSSION

The experiments reported here provide direct evidence that glutamine synthetase in avian liver mitochondria, and probably that in other uricotelic birds (3), represents the primary detoxication step for ammonia generated during hepatic amino acid catabolism and that this enzyme in these species is thus physiologically equivalent to carbamyl phosphate synthetase I in uroteres. The latter enzyme, in combination with ornithine transcarbamylase, is responsible for the intramitochondrial synthesis of citrulline. The two mitochondrial detoxication mechanisms, citrulline or glutamine synthesis, appear to be quantitatively similar in mammals and birds. For example, the rate of glutamine synthesis found here with intact chicken liver mitochondria (0.18 µmol/hour/mg of protein) is com-
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TABLE I

Fragment Analysis of PTH-derivatives of glutamine by high resolution mass spectrometry

<table>
<thead>
<tr>
<th>Synthetic PTH-derivative of glutamine standards</th>
<th>PTH-derivative of glutamine from mitochondrial incubation*</th>
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<tr>
<td>[α-amino-15N]</td>
<td>[amide-15N]</td>
</tr>
<tr>
<td>Mass</td>
<td>Δ</td>
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<tr>
<td>(M+)</td>
<td>Found</td>
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<tr>
<td></td>
<td>Calculated*</td>
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<tr>
<td>(M-17)+</td>
<td>Found</td>
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<td>Calculated</td>
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<tr>
<td>(M-59)+</td>
<td>Found</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>(CH₂CONH₂)</td>
<td>Found</td>
</tr>
<tr>
<td></td>
<td>Calculated</td>
</tr>
</tbody>
</table>

*With [15N]glutamate. See "Experimental Procedures."
*The calculated mass values are from the atomic weights given by König et al. (15).

TABLE II

Uptake of L-glutamine by avian liver mitochondria

Mitochondria were incubated for 1 min at 25° with L-[U-14C]glutamine in 1.0 ml of a reaction mixture containing 10 mM Tris-chloride buffer, pH 7.4; 0.5 mM ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid; 25 mM sucrose; 66.7 mM potassium chloride; and 1.3 μM rotenone. The data represent the mean of four experiments in which the specific radioactivity of the [14C]glutamine was either kept constant or varied. The mitochondrial protein concentrations and corresponding specific radioactivity or total radioactivity in the experiments were as follows: 57.75 mg, 0.01 μCi/μmol; 5.84 mg, 0.009 μCi/μmol; 5.45 mg, 0.8 μCi; 5.9 mg, 0.1 μCi. Following incubation, the reaction mixtures were rapidly centrifuged and the resulting pellets were solubilized and counted as described under "Experimental Procedures." The nonmatrix space was taken as that permeable to [14C]sucrose and the values presented are corrected for the amount of [14C]glutamine present in this space. Matrix volume was calculated by subtracting the [14C]sucrose-permeable space from the H₂O-permeable space (16).

<table>
<thead>
<tr>
<th>L-Glutamine concentration</th>
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<tr>
<td>mM</td>
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<tr>
<td>0.5</td>
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<tr>
<td>1.0</td>
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<tr>
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<td>200.0</td>
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The calculated mass values are from the atomic weights given by König et al. (15).

Fig. 4. Efflux of L-glutamine from avian liver mitochondria. Mitochondria (6 mg of protein/ml) were preloaded by incubation for 4 min in the mixture described in Table II containing 100 mM L-[U-14C]glutamine (specific radioactivity, 0.008 μCi/μmol). The loaded mitochondria were recovered by centrifugation and resuspended in a similar but glutamine-free isotonic potassium chloride medium at 25°. After the times indicated, the samples were centrifuged and the pellets were solubilized and counted as described in the text.

However, be limiting to either citrulline or glutamine synthesis. Based on a kinetic study of glutamate transport into rat liver mitochondria, Bradford and McGivan (20) have suggested that glutamate deamination, and hence the synthesis of citrulline, may be limited by glutamate influx. They report rates of glutamate transport by rat liver mitochondria of between 0.3 and 0.6 μmol/hour/mg of protein. These rates will account for the rate of urea synthesis by the perfused mammalian liver with alanine as a substrate (21). In other experiments, we have obtained rates of glutamine synthesis by avian mitochondria as high as 0.5 μmol/hour/mg of protein. Since only approximately one-half of the glutamate taken up by these mitochondria is oxidatively deaminated, were glutamate transport-limiting, the rates of glutamine synthesis observed would thus require rates of glutamate transport of from 0.36 to 1 μmol/hour/mg of protein.
Another similarity between the avian and mammalian systems is the ease of penetration of the mitochondrial membranes by the two products, glutamine and citrulline. As shown here for glutamine and by Gamble and Lehninger (18) for citrulline, both products rapidly traverse their respective mitochondrial membranes by an apparently passive and energy-independent process. The implied passive exit of the two compounds is in contrast to what is known of the entry of their precursors, glutamate and ornithine. Glutamate entry into rat liver mitochondria occurs via a carrier-mediated hydroxyl or aspartate exchange mechanism (22-24) whereas ornithine entry is via a respiration-dependent transport system (18). Gamble and Lehninger (18) suggest that the translocation of citrulline might be mediated by a neutral amino acid transport system which may or may not be present in mammalian liver mitochondria (25, 26). The titration curves of citrulline and glutamine are identical with that of glycine and the two compounds cannot be separated by electrophoresis at any pH between 6 and 8. The R chains of glutamine and citrulline are thus unprotonable at physiological pH values and any ionization of the molecules are due to the α-amino and carboxyl functions. They would thus seem to be potential substrates for the neutral transport system whose K_m for L-leucine has been reported to be around 0.25 mM (25). Our initial observations on glutamine influx into avian liver mitochondria (Table II) nevertheless indicate that the entry of this compound into these mitochondria is not carrier-mediated, at least not by one saturable between 0.5 and 200 mM external glutamine concentration. The physiological direction of glutamine (and citrulline) movement is, however, outward and, although this is a rapid process (Fig. 4), no observations on the nature of the translocation in this direction have been made.

The most significant similarity between the two mitochondrial detoxication mechanisms may well be the lack of protonization of the R chain-NH_2 function of the products citrulline and glutamine as well as the mitochondrial membrane permeability properties of these two compounds. It is generally felt that the major physiological role of carbamyl phosphate synthetase I in ureotelic liver mitochondria is to rapidly remove ammonia generated via glutamate dehydrogenase and thus pull this reaction in the thermodynamically unfavorable direction of glutamate oxidation (e.g., see Ref. 27). Our interpretation of the data presented in Fig. 1 is based on a similar role for glutamine synthetase in avian liver mitochondria. However, removal of other products of the glutamate dehydrogenase reaction, NADH and α-ketoglutarate, should have the same effect and it would appear that at least some glutamate deamination can proceed in the presence of relatively high initial intramitochondrial ammonia concentrations (24). These can be calculated to be between 11 and 14 mM compared with a K_m for ammonia of mammalian carbamyl phosphate synthetase I of around 1 mM (28). In experiments described by LaNoue and Tischler (24), coupled mitochondria do not release ammonia generated via glutamate oxidation due to an influx of H^+ associated with a transmembrane glutamate-aspartate exchange and the subsequent formation of the impermeant, NH_4^+. Uncoupling, which abolishes H^+ influx, allows the release of ammonia. The applicability in vivo and physiological significance of these results are not known. The action of the two ammonia detoxication mechanisms would, in any event, provide a neutral form of ammonia for efflux from the mitochondria by converting it to either the amide function of glutamine or ureido function of citrulline. Ammonia itself is known to equilibrate protons across both the mitochondrial (29) and chloroplast (30) membrane and thereby uncouple phosphorylation. This would thus not occur with either glutamine or citrulline. With respect to the proposed role of the liver mitochondrial ammonia detoxication mechanisms, it is interesting to note that glutamine synthetase in plants has also recently been localized within the chloroplast (31) and one of the suggested functions of the enzyme in these organelles is the "detoxication" of either exogenous or endogenous ammonia.

Acknowledgment—We are deeply indebted to Dr. Dominic M. Desiderio of the Institute for Lipid Research, Baylor College of Medicine, for his help with the mass spectrographic analyses of the PTH-derivatives of glutamine.

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