The Role of Mitochondrially Bound Arginase in the Regulation of Urea Synthesis

STUDIES WITH [U-15N4]ARGININE, ISOLATED MITOCHONDRIA, AND PERFUSED RAT LIVER*

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The main goal of the current study was to elucidate the role of mitochondrial arginine metabolism in the regulation of N-acetylglutamate and urea synthesis. We hypothesized that arginine catabolism via mitochondrial arginase augments ureagenesis by supplying ornithine for net synthesis of citrulline, glutamate, N-acetylglutamate, and aspartate. [U-15N4]arginine was used as precursor and isolated mitochondria or liver perfusion as a model system to monitor arginine catabolism and the incorporation of 15N into various intermediate metabolites of the urea cycle. The results indicate that 8% of total mitochondrial arginase activity is located in the matrix, and 90% is located in the outer membrane. Experiments with isolated mitochondria showed that 60–70% of external [U-15N4]arginine catabolism was recovered as [15N]aspartate, glutamate, N-acetylglutamate, citrulline, and aspartate. The production of [15N]-labeled metabolites was time- and dose-dependent. During liver perfusion, urea containing one (Um+1) or two (Um+2) 15N was generated from perfusate [U-15N4]arginine. The output of U_m+2 was between 3 and 8% of total urea, consistent with the percentage of activity of matrix arginase. Um+1 was formed following mitochondrial production of [15N]glutamate from [α,6-15N]ornithine and transamination of [15N]glutamate to [15N]aspartate. The latter is transported to cytosol and incorporated into argininosuccinate. Approximately 70, 75, 7, and 5% of hepatic ornithine, citrulline, N-acetylglutamate, and aspartate, respectively, were derived from perfusate [U-15N4]arginine. The results substantiate the hypothesis that intramitochondrial arginase, presumably the arginase-II isozyme, may play an important role in the regulation of hepatic ureagenesis by furnishing ornithine for net synthesis of N-acetylglutamate, citrulline, and aspartate.

1-L-Arginine and/or its metabolites are essential to several metabolic and physiological functions (1–5). Supplementation of arginine to patients with inflammatory disease, trauma, surgery, or tumor may help maintain normal physiologic functions (1–5). Supplementation of arginine to patients with inflammatory disease, trauma, surgery, or tumor may help maintain normal physiologic functions (1–5). Arginine may mediate its biological actions via nitric oxide-dependent and -independent pathways (5). Furthermore, arginine may regulate whole body nitrogen homeostasis following its up-regulation of hepatic N-acetylglutamate (NAG)* synthesis, and thereby ureagenesis (6–11). However, as yet the mechanism(s) underlying the up-regulation of NAG is not completely understood. The liver is a major site for the uptake and metabolism of dietary arginine. Therefore, identification in the liver of the fate of arginine supplementation would enable understanding of the role of this amino acid in the regulation of NAG and urea synthesis as well as its action in normal and abnormal states.

In the urea cycle, arginine synthesis is balanced by arginine catabolism via the cytosolic arginase-I reaction. Thus, the hepatic urea cycle is not involved in the net synthesis or the net catabolism of arginine. Prior studies suggest that there is no equilibrium between dietary arginine and arginine formed in the urea cycle (12, 13). Arginine entering the liver via the portal vein may be metabolized by four sets of enzymes: arginase, nitric-oxide synthase, arginine:glycine amidinotransferase, and arginine decarboxylase (14). Although arginine: glycine amidinotransferase can produce ornithine from arginine (14), arginase is the primary enzyme for generation of ornithine (5, 14–18).

Ornithine is the key intermediary metabolite linking exogenous arginine with urea synthesis (13–18). Thus, the catabolism of arginine via arginase may regulate urea synthesis by furnishing ornithine for synthesis of citrulline in the mitochondrial matrix. It has been shown that isolated mitochondria have a significant amount of arginase bound to the outer membrane (11, 13). ~90% of the mitochondrial bound arginase was removed after washing of isolated mitochondria with 150 mM KCl (11). The remaining 10% of total arginase activity is present in the intramitochondrial matrix or membrane (11). More recent studies have identified this intramitochondrial arginase (IM-arginase) as the arginase-II isozyme (16, 19–22). Arginase-II has a wide tissue distribution and may be involved in biosynthetic functions such as the formation of ornithine, glutamate, and polyamines (16, 19–21). As yet, the extent of arginine hydrolysis to urea and ornithine via IM-arginase and its role in the regulation of NAG and ureagenesis are uncertain. In the current study we have examined the possibility that the IM-arginase is functionally advantageous for the synthesis of urea by providing ornithine for the synthesis of NAG and citrulline.

As illustrated in Fig. 1, we proposed that the mitochondrial

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1 The abbreviations used are: NAG, N-acetylglutamate; GC-MS, gas chromatography-mass spectrometry; IM-arginase, intramitochondrial arginase; MPE, mol % excess; OAT, ornithine aminotransferase.
catabolism of arginine via the IM-arginase reaction initiates a metabolic cascade that includes the formation of ornithine and thereby glutamate as a precursor for both the synthesis of aspartate and NAG, an obligatory activator of carbamyl-phosphate synthetase-I (23). Aspartate would be transported into the cytosol to support the synthesis of argininosuccinate. Hence, the proposed metabolic cascade would result in increased: (i) availability of ornithine for mitochondrial synthesis of NAG and citrulline and (ii) availability of aspartate for cytosolic synthesis of argininosuccinate. This possibility is in accord with the notion that under physiological conditions ureagenesis depends upon mitochondrial ornithine availability and the transport of ornithine into hepatic mitochondria (24–26). This is especially true when mitochondrial ornithine uptake is inhibited by other amino acids (26) or when the mitochondrial ornithine carrier is affected by H+ (27, 28).

Urea genesis begins in mitochondria and finishes in the cytosol (18). Therefore, in the current studies we investigated hepatic arginine metabolism in isolated mitochondria and in a liver perfusion system. Experiments with isolated mitochondria and/or sub mitochondrial fractions provide valuable information concerning the location of arginase within the mitochondrion, its kinetic parameters, mitochondrial arginine metabolism, and the incorporation of its nitrogen into intermediates of the urea cycle (Fig. 1). Perfusion of the structurally intact liver with physiological concentrations of [U-15N4] arginine and other metabolites would demonstrate the incorporation of the perfused 15N-labeled arginine into intermediates of the urea cycle and the production of 15N-labeled urea isotosomers as illustrated in Fig. 1.

The possibility of hepatic zonation (29) is important in terms of the current hypothesis. It is not known whether arginase activity is located exclusively in the perportal hepatocytes, the site of urea synthesis, or may be present also in perivenous hepatocytes, the site of hepatic glutamine synthesis (29). If ornithine, the product of the arginase reaction, furnishes intermediates and/or activator (i.e. NAG) for urea synthesis, one would expect that the IM-arginase and the OAT reaction are located in close proximity to the zone where urea synthesis takes place. To address this question, separate perfusions were carried out with antegrade or retrograde flow, as previously indicated by Häussinger (29) and more recently by Brosnan and co-workers (30, 31).

We used [U-15N4]arginine as a precursor and gas chromatography-mass spectroscopy (GC-MS) methodology to determine: 1) the time course of 15N-labeled arginine catabolism in isolated mitochondria and, consequently, the incorporation of 15N into glutamate, aspartate, citrulline, and NAG; 2) the dependence of 15N-labeled metabolites production on arginine concentration; and 3) the relative uptake and catabolism of perfused [U-15N4]arginine and thereby the production of 15N-labeled metabolites and urea isotosomers by perportal hepatocytes (antegrade perfusion) or perivenous hepatocytes (retrograde perfusion).

The results demonstrate that in isolated mitochondria and liver perfusions 15N-labeled ornithine, glutamate, citrulline, NAG, and urea were formed from external 1-15Nlabeled arginine. The data suggest that the catabolism of arginine via the IM-arginase augments urea synthesis by furnishing the intermediary metabolites necessary for urea synthesis.

EXPERIMENTAL PROCEDURES

Materials and Animals—Male Sprague-Dawley rats (Charles River) were fed ad lib a standard rat chow diet. The chemicals were of analytical grade and were obtained from Sigma-Aldrich. Enzymes and cofactors for the analysis of urea, lactate, pyruvate, and ammonia were obtained from Sigma. 1-[(Guanidino-15N2)arginine, 99 mol% excess (MPE), was from Isotec, and 1-15Nlabeled arginine (99 MPE) was from ICN.

Preparation of Rat Liver Mitochondria and Mitochondrial Matrix—Mitochondria were isolated from the livers of overnight fasted rats by differential centrifugation as previously described (32). Briefly, the liver of an anesthetized rat was cannulated through the portal vein, rinsed with 0.9% NaCl solution (4°C), excised, and weighed. The minced liver was homogenized in a glass Potter-Elvehjem homogenizer with a Teflon pestle in 12.5 volumes of cooled isolation buffer consisting of 225 mM mannitol, 75 mM sucrose, 1 mM EGTA, 5 mM Hepes, pH 7.4. The mitochondrial pellet was gently resuspended with isolation buffer to yield 50–80 mg protein/ml. All of the manipulations were performed in a cold room, and the mitochondrial suspension was kept in an ice bath.

In a separate series of experiments, the mitochondrial pellet was washed two times with 150 mM KCl to remove bound to the outer membrane of mitochondria as previously described (11) and then with isolation buffer to remove any residue of KCl. Separate preparation of KCl-washed mitochondria was used to obtain mitochondrial matrix. To this end, the KCl-washed mitochondrial pellet was resuspended in basic incubation medium (as indicated below) and subjected to three cycles of freezing in liquid nitrogen and thawing, as described previously (33). Mitochondrial matrix following centrifugation for 20 min at 40,000 × g (33). Matrix was removed and added to the incubation flasks (as indicated below).

Metabolic Studies with Isolated Mitochondria—Mitochondrial suspension (3–4 mg protein/ml) was incubated in Erlenmeyer flasks (final volume, 2 ml) at 30°C, in a shaking water bath for the times with the addition of 15N-labeled arginine, as indicated below. The basic incubation medium consisted of 50 mM Tris, 5 mM KCl, 5 mM MgCl2, 15 mM KHCO3, 5 mM KH2PO4, 1 mM NaHCO3, 1 mM α-ketoglutarate, 5 mM succinate, 2 mM ATP, 2 mM octanoic acid, 0.25 mM CoA, and 1 mM Mn2+, pH 7.4.

In the first series of experiments we assessed the percentage of arginine activity in KCl-washed and unwashed mitochondria, KCl-containing supernatants, or the matrix of KCl-washed mitochondria. Isolated mitochondria were carried out for 10 min at 30°C with basic incubation medium containing 10 mM [guanidino-15N2]arginine. Then a series of experiments was performed to determine the Vmax of the flux through the arginase reaction and the Km for arginine in KCl-washed and unwashed mitochondria. To this end, incubations were carried out for 7–10 min (the initial, linear stage of 15Nlabeled urea formation) at 30°C, with increasing concentrations (0–20 mM) of [guanidino-15N2]arginine. Determination of the time course of 15N-labeled arginine catabolism and the incorporation of 15N into other metabolites, the experiments were performed with KCl-washed and unwashed mitochondria. The incubations were carried out with basic medium and 2 mM 1-15Nlabeled arginine. This [Arg] is similar to that found in mitochondria isolated from livers obtained from overnight fasted rats (34). To establish the dependence of intermediate metabolites production on arginine concentration, mitochondrial incubations were carried out for 20 min with basic medium and with increasing concentrations (0–5 mM) of [U-15N4]arginine. At the indicated time, an aliquot (100 μl) was taken for protein determination, and incubation was stopped with 100–150 μl of HClO4 (80%). Metabolite measurements were done in neutralized extracts. Three to five independent experiments were carried out for each series.

Measurement of Mitochondrial Respiration—Respiratory control and oxygen consumption were determined in each mitochondrial preparation as previously described (34). Oxygen consumption usually was 2–3, 9–12, and 2–3 nmol of O2/min/mg of protein for states 2, 3, and 4, respectively, and the VJ/VV or VJ/VV ratio was between 3 and 4. Metabolic studies were carried out with mitochondria having a VJ/VV ratio greater than 3.

Experiments with Liver Perfusions—Livers from overnight fasted male rats were perfused in the nonrecirculating mode as previously described (12, 35). We employed either antegrade or retrograde flow at the rate of 3–3.5 ml/kg liver. The basic perfusion medium was Krebs saline (pH 7.4), continuously gassed with 95% O2, 5% CO2 and containing lactate (2.1 mM) and pyruvate (0.3 mM) as metabolic fuels. pO2 (in influent and effluent media) was monitored throughout, and oxygen consumption was calculated.

The first series of antegrade or retrograde single-pass perfusion was carried out with physiological concentrations of arginine and NH4Cl as nitrogen sources. After 20 min of preperfusion, the basic perfusion medium was replaced by one that contained, in addition to the lactate and pyruvate, 0.3 mM unlabelled NH4Cl and 0.25 mM 1-15Nlabeled arginine. Subsequently, a separate series of antegrade or retro-
Regulation of Hepatic Ureagenesis

**FIG. 1.** The metabolic network of arginine catabolism in cytosolic and mitochondrial compartments and its putative coupling to ureagenesis. Metabolic reactions are: arginase-II (circle 1), OAT (circle 2), Δ-pyrroline-5-carboxylate dehydrogenase (PSCDH) (circle 3), glutamate-aspartate aminotransferase (circle 4), NAG synthetase (circle 5), carbamyl-phosphate synthetase-I (circle 6), ornithine transcarbamylase (circle 7), glutamate dehydrogenase (circle 8), β-oxidation (circle 9), arginase-I (circle 10), nitric-oxide synthase (circle 11), ornithine decarboxylase (circle 12), and phosphate-dependent glutaminase (circle 13). ARS, argininosuccinate; CP, carbamyl-phosphate; α-Kg, α-ketoglutarate; ORNT 1, ornithine/citrulline exchanger (transporter). α, the location of 15N.

1. grade perfusions was carried out with 0.25 mM [U-15N4]arginine, 1 mM unlabeled glutamine, and 0.3 mM NH4Cl. In each of the experiments outlined above, the perfusion was continued for 50 min. Samples were taken from the influent and effluent media for chemical and GC-MS analyses. At the end of the perfusion, the liver was freeze-clamped with aluminum tongs precooled in liquid N2. The frozen liver was ground into a fine powder, extracted into perchloric acid, and used for metabolite determination and 15N GC-MS measurements of 15N isotopic enrichment were performed by isotope dilution as indicated for NAG. Data obtained from mitochondrial extracts were determined using GC-MS and an isotope dilution approach (12). In a few cases samples were spiked with 15N-labeled NAG, and the NAG concentration was calculated as indicated (40). The formation of 15N-ammonia was determined as previously described (41). The concentration of amino acids was determined by high pressure liquid chromatography, utilizing precolumn derivatization with o-phthalaldehyde (42). The levels of ammonia and urea were measured (12, 35). However, we have found that the colorimetric measurement of urea with phenol and hypochlorite is not sufficiently sensitive for determination of a low urea level in experiments with isolated mitochondria. Therefore, after the determination of the initial 15N enrichment in urea (I1), as indicated above, an aliquot (50 μl) of mitochondrial extract was spiked with unlabeled urea, and a second (I2) GC-MS measurement of 15N enrichment was performed. The concentration of urea was calculated by isotope dilution as indicated for NAG. The concentration and 15N enrichment in N-acetylg glutamate in liver or mitochondrial extracts were determined using GC-MS and an isotope dilution approach (12). In a few cases samples were spiked with 15N-labeled NAG, and the NAG concentration was calculated as indicated (40). The formation of 15N-ammonia was determined as previously described (41). The concentration of amino acids was determined by high pressure liquid chromatography, utilizing precolumn derivatization with o-phthalaldehyde (42). The levels of ammonia and urea were measured (12, 35). However, we have found that the colorimetric measurement of urea with phenol and hypochlorite is not sufficiently sensitive for determination of a low urea level in experiments with isolated mitochondria. Therefore, after the determination of the initial 15N enrichment in urea (I1), as indicated above, an aliquot (50 μl) of mitochondrial extract was spiked with unlabeled urea, and a second (I2) GC-MS measurement of 15N enrichment was performed. The concentration of urea was calculated by isotope dilution as indicated for NAG. Calculations and Statistical Analyses—Data obtained from mitochondrial incubations were analyzed with GraphPad Prism 4 software for linear and nonlinear curve fitting. Catabolism of [U-15N4]arginine during the course of the incubation was fitted to a single exponential decay Y = I0 - Ie e^(-kt), where, I0 represents the time 0 intercept and Ie represents the concentration of [U-15N4]arginine at the end of the incubation, respectively. The rate (nmol/min/mg protein) of [U-15N4]arginine catabolism (Ie) was calculated by the product of U0 –
The production of [15N]urea from [U-15N4]arginine was used to calculate the flux through arginase ($Q_a$). In most cases, the rate of 15N-labeled metabolites production ($MP_{15N}$) was fitted to a one-phase exponential association ($Y = Y_{\text{max}} \times (1 - e^{-kt})$), and the production rate (nmol/min/mg protein) of each metabolite was calculated by the product of the corresponding $Y_{\text{max}} \times k$. In a few cases the production of 15N-labeled metabolites was best fitted to a linear regression analysis, and the production of 15N-labeled metabolites was determined from the slope of the regression lines. The GraphPad Prism 4 software was also used to determine the best curve fit and to calculate the $V_{\text{max}}$ for the flux through arginase and the production of 15N-labeled metabolites as well as the $K_m$ for arginine in experiments with an increasing concentration of arginine.

During liver perfusions, the rate of precursor-N uptake or the output of metabolites was determined by the measurement of metabolite concentration in the influent and effluent (nmol/ml), normalized to the flow rate (ml/min) and liver wet weight (35). The output of 15N-labeled metabolites was calculated by the product of 15N enrichment (MPE/100) times concentration (nmol/min/g wet wt) and is expressed as nmol 15N metabolite/min/g wet wt. The rate of perfusate arginine catabolism via the arginase reaction is represented by the output in the effluent of [15N2]urea during the course of perfusion with 15N-labeled arginine. The percentage of incorporation of [U-15N4]arginine into 15N-labeled product (15N product) was calculated as [15N product (MPE/[U-15N4]arginine (MPE))] × 100, using 15N enrichment in the freeze-clamped liver at the end of each perfusion.

Each series of experiments was repeated three to five times with different mitochondrial preparations or three to four times with liver perfusion as outlined above. Statistical analysis was carried out using In-STAT 1.14 software for the Macintosh. A Student’s $t$ test or an analysis of variance test was employed to compare two groups or differences among groups as needed. A $p$ value less than 0.05 was taken as indicating a statistically significant difference.

RESULTS AND DISCUSSION

An important feature of the current studies is the use of [U-15N4]arginine as a tracer to evaluate hepatic arginine metabolism and in particular the incorporation of 15N into intermediar metabolites of the urea cycle by isolated mitochondria or a liver perfusion system. The sensitivity and precision of GC-MS make it a superb methodology to identify the primary metabolites of [U-15N4]arginine and their role in the regulation of urea synthesis, an advantage we have utilized in our previous studies dealing with the regulation of hepatic ureagenesis (12, 35–37, 41).

Prior studies proposed that arginine acts as an allosteric activator of mitochondrial N-acetylglutamate synthetase (18). However, this concept has been the subject of disagreement (6–11, 43, 44). Recently, we have proposed that agmatine, the product of the arginine decarboxylase reaction, may augment NAG synthesis and thereby urea synthesis (12). In the current investigation we have explored an alternative, but not mutually exclusive, possibility. As illustrated in Fig. 1, we propose that arginine entering the liver via the portal vein is metabolized by IM-arginase to form ornithine and urea. Subsequently, ornithine serves as substrate for glutamate and citrulline synthesis. Glutamate so formed is then used for the synthesis of NAG and/or is transaminated to form aspartate for the synthesis of arginosuccinate. Taken together, the current results support the hypothesis that mitochondrial arginine metabolism up-regulates ureagenesis by furnishing ornithine for net synthesis of NAG, citrulline, and aspartate. The following findings bear directly on this mechanism of arginine stimulation of hepatic urea synthesis.

Characterization of Mitochondrially Bound Arginase—The initial series of experiments was designed to determine the activity of arginase in submitochondrial fractions. In KCl-washed mitochondria, unwashed mitochondria, mitochondrial matrix of KCl-washed mitochondria, and KCl-containing supernatant, arginase activity was 11.4, 94, 7.1, and 87 nmol/min/mg protein, respectively. In unwashed mitochondria, the half-maximal activity of arginase ($K_{m}$) was achieved with [a-arginine] at 10.4 ± 1.1 mm with a $V_{\text{max}}$ of 657 ± 24 nmol/min/mg protein. In KCl-washed mitochondria the $K_{m}$ value was 1.27 ± 0.07 mm with a $V_{\text{max}}$ of 34 ± 0.5 nmol/min/mg protein. These values are in good agreement with the prior study of Cheung and Rajima (11). The current study demonstrates that ~90% of mitochondrial bound arginase is located in the outer membrane, and 8% is in the mitochondrial matrix. Furthermore, the arginase activity in KCl-washed mitochondria is approximately that of the activity in isolated matrix. Thus, the arginase bound to the outer mitochondrial membrane presumably represents the activity of the arginase-I isozyme, whereas arginase in KCl-washed mitochondria, which is mainly located in the matrix, probably represents the activity of the arginase-II

![FIG. 2. Time course of [U-15N4]arginine catabolism and formation of 15N-labeled urea and ornithine. The experiments were carried out with 2 mM [U-15N4]arginine and with KCl-washed (A) or unwashed mitochondria (B). A, time course of unidirectional catabolism of [U-15N4]arginine. B, production of 15N-labeled urea. C, production of [α,δ-15N4]ornithine. The lines represent the best fits of the data to a single exponential-decay for [U-15N4]arginine disappearance and first order kinetic ($Y = Y_{\text{max}} \times (1 - e^{-kt})$) for 15N-labeled urea and Orn production during the course of the incubation. The bars are the means ± S.D. of three to five independent experiments.](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2704173/fig2.png)
isozyme. This conclusion is in agreement with numerous studies indicating that the cytosolic arginase-I isozyme is located near the outer mitochondrial membrane (11, 13, 43), and the arginase-II isozyme is primarily intramitochondrial (19–22).

Mitochondrial Catabolism of [U-15N4]Arginine and Production of 15N-Labeled Metabolites—Following the demonstration that arginase activity is present in the mitochondrial matrix, we next sought to determine the time course of [U-15N4]arginine catabolism and incorporation of 15N into various metabolites in KCl-washed and unwashed mitochondria. Data for this series of experiments are presented in Figs. 2 and 3 and Table I.

In KCl-washed and unwashed mitochondria, the catabolism of [U-15N4]arginine was best fitted to a single exponential decay curve ($r^2 = 0.99$; Fig. 2A). The ratio between the catabolism rate ($C_r$) of [U-15N4]arginine in KCl-washed and unwashed mitochondria was $-0.08$ (Table I), in good agreement with matrix arginase activity as a fraction of total mitochondrially bound arginase. The production of 15N-labeled urea and ornithine was inversely correlated to the disappearance of [U-15N4]arginine (Fig. 2). In KCl-washed mitochondria, 15N-labeled urea and ornithine increased almost linearly throughout the course of the incubation. In unwashed mitochondria, 15N-labeled urea and ornithine increased linearly up to 7–10 min and then reached a plateau between 10 and 30 min (Fig. 2).

The $T_{1/2}$ values of arginase catabolism in experiments with KCl-washed and unwashed mitochondria were $-17$ and 2 min, respectively. Hence, the plateau production of 15N-labeled urea and ornithine in unwashed mitochondria was probably due to the consumption of [U-15N4]arginine during the first 5 min of incubation.

FIG. 3. Time course of 15N-labeled metabolites production by isolated mitochondria incubated with [U-15N4]arginine. The experiments were carried out with 2 mM [U-15N4]arginine and with KCl-washed (•) or unwashed mitochondria (○), as detailed under “Experimental Procedures.” A, production of [15N]glutamate. B, production of [15N]aspartate. C, production of [15N]acetylglutamate. D, production of [α,δ-15N2]citrulline. The lines represent the best fits of the data to one phase exponential association or linear regression analysis. The bars are the means ± S.D. of three to five independent experiments.
NAG synthesis, a finding confirmed in experiments with $^{13}$C-glutamate synthetase (Fig. 3). Mitochondria $^{15}$N enrichment data demonstrate a precursor line, aspartate, and NAG, in both KCl-washed and unwashed mitochondria. $^{15}$N-labeled ornithine, glutamate, citrulline was recovered as $^{15}$N-labeled ornithine, glutamate, citrulline. Thus, the current observations, together with earlier data (6–13, 25, 26, 43, 46), demonstrate that mitochondrial oxidation of arginine provides ornithine and glutamate to the incubation (Fig. 2A). Because ornithine serves as an intermediary metabolite and is further metabolized in mitochondria (Fig. 1), the formation of $^{15}$N-labeled asparagine, an end product, was used to calculate the flux through the arginase reaction ($Q_A$). In KCl-washed and unwashed mitochondria, the values of $Q_A$ are 9.6 and 144 nmol/min/mg protein, respectively (Table I). The ratio between $Q_A$ values in experiments with KCl-washed and unwashed mitochondria is ~0.07, in good agreement with the percentage of matrix arginase of the total mitochondrially bound arginase velocity.

Table I indicates that ~60–70% of $[{U}^{15}$N$]_{arginine}$ catabolism was recovered as $^{15}$N-labeled ornithine, glutamate, citrulline, aspartate, and NAG, in both KCl-washed and unwashed mitochondria. $^{15}$N enrichment data demonstrate a precursor-product relationship between $[\alpha,\delta^{15}$N$]_{ornithine}$ (precursor) and $[\alpha,\delta^{15}$N$]_{citrulline}$ or between $[\alpha,\delta^{15}$N$]_{ornithine}$ and $[\alpha^{15}$N$]_{glutamate}$, indicating that $[\alpha,\delta^{15}$N$]_{ornithine}$, the intermediary metabolite of $[{U}^{15}$N$]_{arginine}$, was further metabolized to form $^{15}$N-labeled citrulline and glutamate. As illustrated in Fig. 1, $[\alpha,\delta^{15}$N$]_{citrulline}$ must have been formed from $[\alpha,\delta^{15}$N$]_{ornithine}$ and unlabeled carbamyl-phosphate via the ornithine transcarbamylase reaction, and $[\alpha^{15}$N$]_{glutamate}$ must have been formed via the urea cycle reaction. $^{15}$N-Glutamate was transaminated to form $^{15}$N-aspartate (Fig. 3B). The data indicate a rapid equilibrium between $^{15}$N-labeled glutamate and aspartate. The production of aspartate in mitochondria and its translocation into cytosol may serve as a major source for argininosuccinate synthesis, consistent with a previous study using a liver perfusion system (35).

$^{15}$N-Glutamate generated from $[\alpha,\delta^{15}$N$]_{ornithine}$ also was used to synthesize $^{15}$N-labeled NAG by mitochondrial N-acetylglutamate synthetase (Fig. 3C). The addition to the medium of octanoic acid and CoA provided most acetyl-CoA required for NAG synthesis, a finding confirmed in experiments with $^{13}$C-labeled octanoic acid (data not shown). In KCl-washed mitochondria, the production of $^{15}$N-labeled NAG shows a lag period of ~5–7 min and then a linear increase during the course of the incubation (Fig. 3C). In unwashed mitochondria, the production of $^{15}$N-labeled NAG linearly increased between 0 and 20 min and then reached a plateau. In both KCl-washed and unwashed mitochondria, the production of $^{15}$N-labeled NAG (Fig. 3C) was linearly correlated to the production of $^{15}$N-glutamate (Fig. 3A), indicating a dependence of NAG synthesis on mitochondrial glutamate.

The mitochondrial level of NAG was ~0.3–0.4 nmol/mg protein. In experiments with unwashed mitochondria, this value was increased to ~1.2–1.5 nmol/mg (sum of $^{15}$N-labeled and unlabeled), after 30 min of incubation with 2 mM $[{U}^{15}$N$]_{arginine}$. Assuming that 1 g wet weight liver contains 60 mg of mitochondrial protein (45), the intramitochondrial NAG (NAG) in the liver would have been ~25 and 80 nmol/g before and at the end of the incubation with 2 mM arginine, respectively. These values are consistent with the NAG concentration of ~76 nmol/g currently found in freeze-clamped liver at the end of perfusion with 0.25 mM $[{U}^{15}$N$]_{arginine}$ and unlabeled glutamine. Furthermore, when considered in the context of the regulation of urea synthesis in vivo, the current data suggest that the mitochondrial NAG and the corresponding rates of $[\alpha,\delta^{15}$N$]_{citrulline}$ synthesis, both in KCl-washed and unwashed mitochondria (Table I), fall within the physiological range and within the range of 4–50 nmol/min/mg previously reported when isolated mitochondria were incubated with ornithine (46). Thus, the current observations, together with earlier data (6–13, 25, 26, 43, 46), demonstrate that mitochondrial arginine catabolism provides ornithine and glutamate to promote NAG and citrulline synthesis. In addition, the current data demonstrate a linear relationship between the production of $^{15}$N-labeled ornithine, NAG, and citrulline during the course of incubations (Figs. 2 and 3) or with an increasing concentration of $[{U}^{15}$N$]_{arginine}$ (Fig. 4), consistent with the notion that the availability of mitochondrial ornithine supports the synthesis of citrulline and thus ureagenesis (24–26, 28, 43, 46).
The synthesis of citrulline was used as proxy for carbamyl phosphate synthesis (10, 25, 45). In both KCl-washed and unwashed mitochondria, the time course of \([\alpha,\delta-15N_2]\)citrulline production was best fitted to a one phase exponential curve, reaching a similar \(Y_{\text{max}}\) after ~20 min of incubation with \([U-15N_4]\)arginine (Fig. 3D). In experiments with unwashed mitochondria, which are similar to those previously described (9), the maximum production of \([\alpha,\delta-15N_2]\)citrulline and thereby carbamyl phosphate occurred when \([15N]\)-labeled arginine was ~150 pmol/mg mitochondrial protein (Fig. 3, C and D). In KCl-washed mitochondria the rate of \([\alpha,\delta-15N_2]\)citrulline production was 2.24 ± 0.2 nmol/min/mg protein, and in unwashed mitochondria it was 7.4 ± 0.8 nmol/min/mg protein (Table I). These values are consistent with a previous study using isolated mitochondria of mouse or rat liver and 2 mM arginine (9). Furthermore, in unwashed mitochondria, the half-maximal synthesis of \([15N]\)-labeled citrulline and NAG was achieved with ~0.11 and 0.47 mM arginine, respectively. The \(V_{\text{max}}\) was 35 and 0.61 nmol/min/mg for citrulline and NAG, respectively (Table II). These values are in agreement with the \(K_m\) and \(V_{\text{max}}\) values obtained when isolated mitochondria were incubated with increasing concentrations of arginine (9). However, previous studies were unable to identify the mechanism(s) by which arginine stimulates NAG synthesis because the earlier investigations did not trace the metabolic fate of arginine, as was done in this study.

![Figure 4](http://www.jbc.org/)

**Fig. 4.** Dependence of \([15N]\)-labeled intermediary metabolite production on the concentration of arginine. The experiments were carried out with KCl-washed (A) or unwashed mitochondria (B). The data were obtained following 20 min of incubation of isolated mitochondria with basic medium and increasing concentrations (0–5 mM) of \([U-15N_4]\)arginine. The observations demonstrate the production in the mitochondria of \([15N]\)-labeled glutamate (A), aspartate (B), N-acetylglutamate (C), and citrulline (D). The lines are the best fits of the data to nonlinear regression analysis (Michaelis-Menten kinetics) using Prism 4 GraphPad Software. The bars are the means ± S.D. of three to five independent experiments.

An observation of special importance is that the \(V_{\text{max}}\) values for \([\alpha,\delta-15N_2]\)citrulline synthesis are about the same in KCl-washed and unwashed mitochondria (Table II). Thus, the catabolism of \([U-15N_4]\)arginine via the IM-arginase reaction (Figs. 3 and 4 and Table II) is sufficient to provide the required ornithine for citrulline synthesis. Therefore, although the IM-arginase isozyme constitutes ~8% of the total mitochondrially bound arginase, it provides an adequate amount of ornithine for intramitochondrial synthesis of NAG and citrulline and thereby may have a key role in the up-regulation of ureagenesis. These observations, together with the time and dose dependence of \([U-15N_4]\)arginine incorporation into NAG, citrulline, and aspartate (Figs. 3 and 4 and Tables I and II), are in line with the hypothesis that mitochondrial arginine metabolism up-regulates ureagenesis by furnishing ornithine for net synthesis of NAG, citrulline, and aspartate.

**Characterization of \([U-15N_4]\)Arginine Catabolism in the Perfused Liver**—To demonstrate the incorporation of \([15N]\)-labeled arginine into intermediates of the urea cycle and the production of \(U_{\text{max}}\) (Fig. 1) in an intact organ, the experiments were carried out with a liver perfusion system. We first employed a series of antegrade or retrograde single-pass perfusions with physiological concentrations of \([U-15N_4]\)arginine (0.25 mM) and unlabeled NH₄Cl (0.3 mM) as a nitrogen source for urea synthesis. A second series of perfusions was performed with the addition of glutamine and ammonia to determine hepatic uptake and catabolism of arginine with an optimal supplementation of nitrogen sources for urea synthesis. Glutamine is a major precursor for urea nitrogen as well as a source of glutamate for NAG synthesis (36, 37). Thus, the addition of unlabeled glutamine would provide information regarding the relationship between the activity of the urea cycle and the metabolism of perfusate \([U-15N_4]\)arginine. Results for this series of experiments are presented in Figs. 5–7.

| Table II: Kinetic parameters of \([15N]\)-labeled metabolite production by isolated mitochondria incubated with increasing concentrations of \([15N]\)-labeled arginine. |
| --- |

| Concentration (mM) | K\(_m\) (mM) | V\(_{\text{max}}\) (nmol/min/mg protein) |
| --- | --- | --- |
| [U-15N_4]arginine | 1.52 ± 0.29 | 23.9 ± 1.9 |
| [U-15N_4]aspartate | 0.59 ± 0.22 | 8.2 ± 0.5 |
| [α,δ-15N_2]citrulline | 0.19 ± 0.02 | 32.7 ± 0.9 |
| [15N]Acetylglutamate | 1.23 ± 0.06 | 0.210 ± 0.004 |

An observation of special importance is that the \(V_{\text{max}}\) values for \([\alpha,\delta-15N_2]\)citrulline synthesis are about the same in KCl-washed and unwashed mitochondria (Table II). Thus, the catabolism of \([U-15N_4]\)arginine via the IM-arginase reaction (Figs. 3 and 4 and Table II) is sufficient to provide the required ornithine for citrulline synthesis. Therefore, although the IM-arginase isozyme constitutes ~8% of the total mitochondrially bound arginase, it provides an adequate amount of ornithine for intramitochondrial synthesis of NAG and citrulline and thereby may have a key role in the up-regulation of ureagenesis. These observations, together with the time and dose dependence of \([U-15N_4]\)arginine incorporation into NAG, citrulline, and aspartate (Figs. 3 and 4 and Tables I and II), are in line with the hypothesis that mitochondrial arginine metabolism up-regulates ureagenesis by furnishing ornithine for net synthesis of NAG, citrulline, and aspartate.

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In the presence of 0.3 mM unlabeled ammonia, the uptake of \([U-15N_4]\)arginine was ~70 nmol/min/g (Fig. 5A). The direction of perfusion did not affect the uptake of arginine nor the flow rate (~3–3.5 ml/min), oxygen consumption (~2.5–3 μmol/min/g), or total urea output (~450–500 nmol/min/g). Similarly, prior studies have shown that arginine metabolism, and thereby urea output and \(1^4\)CO₂ production from perfusate \([U-14C]\)arginine, were the same when livers were perfused in an antegrade or retrograde direction (30). Therefore, from this series of experiments we present only data.
obtained with physiologic flow direction (Fig. 5), which would reflect catabolism of [U-15N4]arginine in periportal hepatocytes and the incorporation of 15N into various metabolites at the site of urea synthesis.

Similarly, in perfusions with glutamine, the uptake of [U-15N4]arginine was 80 nmol/min/g (Fig. 6A), indicating that glutamine did not affect hepatic uptake of arginine. In addition, the direction of perfusion did not affect the uptake of arginine (Fig. 6A) or oxygen consumption (~2.5–3 μmol/min/g). However, total urea output was ~2-fold higher with antegrade (~800 nmol/min/g) versus retrograde (~400 nmol/min/g) perfusions, demonstrating the high capacity of the perportal hepatocytes versus the perivenous hepatocytes in producing urea when glutamine was added to the perfusate.

Figs. 5 and 6 demonstrate an immediate production and output of the Um1 isotopomer. The output of Um1 must have occurred secondary to the catabolism of [U-15N4]arginine to [15N2]urea and [α,δ-15N2]ornithine. With antegrade flow, the enrichment in Um1 was ~3 and 8 MPE in the presence and absence of glutamine in the perfusate, respectively. The rate of Um2 was ~30 nmol/min/g with or without the addition of
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mitochondrial production of ornithine, similar to the channeling between the argininosuccinate lyase and arginase-I reactions (13). The current study demonstrates that IM-arginase abets the synthesis of urea, as illustrated in Fig. 1. This conclusion is strongly supported by the data in Figs. 5–7, which demonstrates the production of [15N]aspartate and U_m+1 during liver perfusion with [U-15N4]arginine.

U_m+1 was formed only during perfusion in the antegrade flow direction (Figs. 5B and 6B), apparently in periportal hepatocytes, the site of urea synthesis. As illustrated in Fig. 1, U_m+1 must have been generated following production of [15N]glutamate from [α, δ, 15N2]ornithine and simultaneous transamination of [15N]glutamate to [15N]aspartate. The latter is translocated to cytosol and used to synthesize argininosuccinate, as demonstrated by the output of U_m+1 (Figs. 5B and 6B). In perfusions without glutamine, enrichment in U_m+1 was ~2 MPE, indicating that ~5% of aspartate required for urea synthesis was derived from perfusate [U-15N4]arginine, and ~95% was from perfusate ammonia and internal sources, including proteolysis as previously suggested (35, 41). In perfusions with unlabeled glutamine, the isotopic enrichment in U_m+1 was ~1 MPE, indicating that in the presence of glutamine and ammonia, ~3% of aspartate utilized for urea synthesis was derived from perfusate [U-15N4]arginine.

Further evidence supporting the above conclusion is provided in Fig. 7, which represents the enrichment data for nitrogen-containing metabolites in freeze-clamped livers at the end of antegrade perfusions. It is evident that, when [U-15N4]arginine was the labeled precursor, 15N was incorporated into glutamate, aspartate, citrulline, and N-acetylglutamate. In perfusions without glutamine (Fig. 7A), the ratio between 15N-labeled ornithine, citrulline, NAG, and aspartate and the precursor, [U-15N4]arginine, indicates that ~10, 75, 8, 7, and 5% of hepatic ornithine, citrulline, glutamate, NAG, and aspartate, respectively, were derived from perfusate [U-15N4]arginine. Similar calculations in perfusions with glutamine (Fig. 7B), indicate that ~70, 65, 5, 3, and 7% of hepatic ornithine, citrulline, glutamate, aspartate, and NAG, respectively, were derived from perfusate [U-15N4]arginine. These values are not significantly different from the values obtained with perfusions without glutamine. Therefore, 0.25 mM perfusate arginine furnished between 7 and 10% of the hepatic NAG and 3–5% of the hepatic aspartate pool, even in the presence of glutamine, the major source of hepatic glutamate and thus NAG (36). Because the production of 15N-labeled NAG and other metabolites was dose-dependent (Fig. 4), the production of NAG and/or aspartate would probably be many fold higher with therapeutic doses of arginine.

The current findings also provide a clue regarding the location of arginase-II and/or the OAT reaction within the liver acinus. Data in Figs. 5 and 6 demonstrate little differences in arginase and OAT activity between liver perfusions with antegrade or retrograde flow. However, the output of U_m+1 was ~2-fold higher with retrograde compared with antegrade flow, suggesting a 2-fold higher activity of arginase-II in perivenous than in periporal hepatocytes. Similarly, 15N-labeled glutamate, NAG, citrulline, and aspartate from perfusate [U-15N4]arginine were generated both in antegrade or retrograde flow, indicating that OAT activity is present in both perivenous and periporal regions. This differs from the finding by Darnell and co-workers (48) suggesting that the OAT mRNA is mainly located in the perivenous hepatocytes where glutamine is formed. In the prior study experiments were carried out with mouse tissue, and the differences may reflect species differences in the expression and/or location of the OAT mRNA. Additionally, the in situ mRNA analysis used by Darnell and co-workers (48) does not

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Fig. 7. Isotopic enrichment in 15N-labeled metabolites in freeze-clamped liver at the end of perfusion. A, freeze-clamped liver at the end of antegrade perfusion with 0.25 mM [U-15N4]arginine and 0.3 mM NH4Cl. B, as in A plus 1 mM unlabeled glutamine. The bars are the means ± S.D. for three livers.

glutamine (Figs. 5B and 6B). Thus, the output of U_m+1 was between 3 and 8% of total urea output. This fraction of U_m+1 of total urea output is in good agreement with the velocity of matrix arginase in KCl-washed mitochondria, suggesting that the IM-arginase reaction may be responsible for generation of U_m+1. If perfusate [U-15N4]arginine equilibrated with arginine formed in the urea cycle, then the enrichment in U_m+1 would be above 50 MPE, similar to the enrichment in [U-15N4]arginine in the freeze-clamped liver extract (Fig. 7). However, the data in Figs. 5–7 demonstrate that perfusate arginine did not equilibrate with arginine formed in the urea cycle.

An important question is whether during liver perfusion the output of U_m+1 represents the activity of arginase-II or a mixture of arginase-I and arginase-II isozymes. The structure and function of both isozymes are nearly identical, and the available inhibitors (i.e. boronic acid, N⁴-hydroxy-L-arginine) act similarly on both (22). Although antibodies against the arginase-I isozyme were previously used to differentiate between arginase-I and arginase-II (47), this approach cannot be used to determine the relative flux through each isozyme during liver perfusion. Thus, the current study cannot definitely determine whether during liver perfusion the production of U_m+1 was solely mediated via the arginase-II reaction. On the other hand, the finding that the fraction of total urea output comprised by U_m+1 (Figs. 5B and 6B) agrees closely with the percentage of activity of matrix arginase suggests that U_m+1 was derived via the IM-arginase reaction. It was proposed that the IM-arginase might be associated with the ornithine transporter in the mitochondrial membrane (28). This arrangement may channel external arginine to arginase-II, thereby providing efficient
necessarily reflect the presence or absence of metabolic reactions as demonstrated here with 15N-labeled arginine and GC-MS methodology. An additional point of interest is that we did not detect 15N-labeled glutamine output during perfusions with [U-15N4]arginine plus unlabeled ammonia, neither with retrograde nor antegrade flow. It is possible that the 15N enrichment in glutamine was below the GC-MS detection limit (−0.5 MPE).

In summary, the current study provides strong evidence to support the hypothesis that arginine stimulates ureagenesis secondary to its catabolism via intramitochondrial arginase, thereby supplying ornithine for synthesis of citrulline, glutamate, NAG, and aspartate. We previously demonstrated that arginine, the product of arginine decarboxylation, stimulated the synthesis of NAG (12). The current findings, demonstrating that arginine furnishes substrates for NAG, citrulline, and aspartate, offer an additional and/or an alternative mechanism by which arginine may stimulate NAG synthesis and thus up-regulate ureagenesis.

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REFERENCES

1. Tapiero, H., Mathe, G., Couvreur, P., and Tew, K. D. I. (2002) Biomed. Pharmacother. 56, 439–445
2. Yeh, C. L. (2004) Nutrition 20, 581–582
3. Wheatley, D. N., and Campbell, E. (2002) Pathol. Oncol. Res. 8, 18–25
4. Reyes, A. A., Karl, I. E., and Klahez, S. (1994) Am. J. Physiol. 267, F331–F346
5. Wu, G., and Morris, S. M. (1998) Biochem. J. 336, 1–17
6. Stewart, P. M., and Walser, M. (1980) J. Biol. Chem. 255, 5270–5280
7. Kamemoto, E. S., and Atkinson, D. E. (1985) Arch. Biochem. Biophys. 243, 100–107
8. Merimote, B. H., Brady, J. F., and Atkinson, D. E. (1990) Biochem. J. 272, 671–675
9. Kawamoto, S., Sonoda, T., Ohtake, A., and Tatibana, M. (1985) Biochem. J. 233, 329–334
10. Beliveau, C. G., Cheung, C.-W., Cohen, N. S., Brusilow, S., and Raijman, L. (1993) Biochem. J. 292, 241–247
11. Cheung, C.-W., and Raijman, L. (1981) Arch. Biochem. Biophys. 209, 643–649
12. Nissim, I., Horyn, O., Daikhin, Y., Nissim Ilana, Lazarow A., and Yudkoff, M. (2002) Am. J. Physiol. 283, E1125–E1134
13. Cheung, C.-W., Cohen, N. S., and Raijman, L. (1989) J. Biol. Chem. 264, 4038–4044
14. Morris, S. M., Jr. (2004) J. Nutr. 134, 2743S–2747S.
15. Morris, S. M., Jr. (1992) Annu. Rev. Nutr. 12, 81–101
16. Morris, S. M., Jr. (2004) Curr. Opin. Clin. Nutr. Metab. Care 7, 45–51
17. Reckowski, R. S., and Ash, D. E. (1994) Arch. Biochem. Biophys. 312, 31–37
18. Meijer, A. J., Lamers, W. H., and Chamuleau, R. A. F. M. (1990) Physiol. Rev. 70, 701–744
19. Cederbaum, S. D., Yu, H., Grody, W. W., Kern, R. M., Yoo, P., and Iyer, R. K. (2004) Mol. Genet. Metab. 82, S38–S44
20. Colleluori, D. M., Morris, S. M., Jr., and Ash, D. E. (2001) Arch. Biochem. Biophys. 389, 135–143
21. Viskelby, J. G., Jenkins, C. P., Shakula, H., Kern, R. M., Grody, W. W., and Cederbaum, S. D. (1996) Genomics 38, 118–123
22. Ash, E. D. (2004) J. Nutr. 134, 2760S–2764S
23. Fahlen, L., Schoder, J. M., Gehred, G. A., and Cohen, P. P. (1964) J. Biol. Chem. 239, 1935–1942
24. McGivan, J. D., Bradford, N. M., and Mendes-Mourao, J. (1976) Biochem. J. 154, 415–421
25. Cohen, N. S., Cheung, C. W., and Raijman, L. (1980) J. Biol. Chem. 255, 10248–10255
26. Naganuma, Y., Hayase, K., and Yoshida, A. (1997) J. Nutr. Sci. Vitaminol. 43, 387–396
27. Indiveri, C., Tonazzi, A., Stipani, I., and Palmieri, F. (1997) Biochem. J. 327, 349–355
28. Camacho, J. A., Obie, C., Biery, B., Goodman, B. K., Hu, C. A., Almashanu, S., Steel, G., Casey, R., Lambert, M., Mitchell, G. A., and Valle, D. (1999) Nat. Genet. 22, 161–168
29. Haussinger, D. (1990) Biochem. J. 267, 281–290
30. O'Sullivan, D., Brosnan, J. T., and Brosnan, M. E. (1998) Biochem. J. 330, 627–632
31. O'Sullivan, D., Brosnan, J. T., and Brosnan, M. E. (2000) Am. J. Physiol. 278, E516–E521
32. Vatamaniuk, M. Z., Horyn, O. V., Vatamaniuk, O. K., and Doliba, N. M. (2003) Biochim. Biophys. Acta 1589, 636–644
33. Szweda, L. I., and Atkinson, D. E. (1989) J. Biol. Chem. 264, 15357–15360
34. Horyn, O., Luhyovy, B., Lazarow, A., Daikhin, Y., Nissim, I., Yudkoff, M., and Nissim, I. (2005) Biochem. J., in press
35. Nissim, I., Horyn, O., Luhyovy, B., Lazarow, A., Daikhin, Y., Nissim, I., and Yudkoff, M. (2003) Biochem. J. 376, 179–188
36. Nissim, I., Yudkoff, M., and Brosnan, J. T. (1996) Biochem. J. 271, 31234–31242
37. Horman, I., and Hesford, F. J., (1974) Biomedical J Mass Spectrometry, 1
38. Marshall, M., and Cohen, P. P. (1972) J. Biol. Chem. 247, 1641–1653
39. Yang, D., Puchowicz, M. A., David, F., Powers, L., Halperin, M. L., and Brunengraber, H. (1999) J. Mass Spectrom. 34, 1130–1136
40. Brosnan, J. T., Brosnan, M. E., Yudkoff, M., Hiss, B. L., Meijer, A. J. (1984) Biochim. Biophys. Acta 802, 497–412
41. Cederbaum, S. D. (1996) Genomics 387–396
42. Meijer, A. J., Lamers, W. H., and Chamuleau, R. A. F. M. (1990) Physiol. Rev. 70, 701–744
The Role of Mitochondrially Bound Arginase in the Regulation of Urea Synthesis: STUDIES WITH [U-15N4]ARGININE, ISOLATED MITOCHONDRIA, AND PERFUSED RAT LIVER
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