Amino Acids (1995) 9: 299–316

Amino
Acids

Metabolism of citrulline in man

Review Article

D. Rabier and **P. Kamoun**

Laboratoire URA CNRS 1335, Université René Descartes
Hôpital Necker – Enfants Malades, Paris, France

Accepted July 1, 1995

Summary. Citrulline is a non protein amino acid involved in three important metabolic pathways, the intrahepatic transformation of ammonia to urea, the de novo synthesis of arginine from glutamine in gut and kidney, the nitric oxide synthesis. The two first pathways use the same enzyme activities but are regulated in different way. This review describe these pathways and their regulation in different tissues. In the light of our knowledge we tried to explain the physiological and pathological (inherited or acquired) variations in man.

Keywords: Amino acids – Citrulline

Introduction

Citrulline is a non protein amino acid formed in the urea cycle pathway by condensation of ornithine and carbamoylphosphate (Fig. 1). Its formation is catalysed by ornithine transcarbamoylase (OTC, EC 2.1.3.3), which is localized in mitochondria of hepatocytes and enterocytes (Jones et al., 1961). Recently a new enzymatic reaction for biosynthesis of citrulline from arginine was described in endothelial cells (Moncada, 1992); this reaction is catalysed by a family of enzymes, known as the nitric oxide synthases. Citrulline formed by the above reactions is used for arginine synthesis in almost all tissues and for urea synthesis in liver tissue only. Citrulline is found as a free-amino acid in plasma and it is present in many other physiological fluids such as urine, cerebrospinal fluid, amniotic fluid and sweat. Its concentration in plasma reflects the activities of the enzymes involved in its synthesis and subsequent utilization in different tissues. Citrulline, like other ∝-amino acids may be measured by ninhydrin coloration after ion-exchange chromatography. Citrulline also has an ureido group $(H_2N—\overset{\overset{O}{\|}}{C}—NH)$ and, after urease treatment of the biological fluids (Kamoun et al., 1983) this chemical group allows

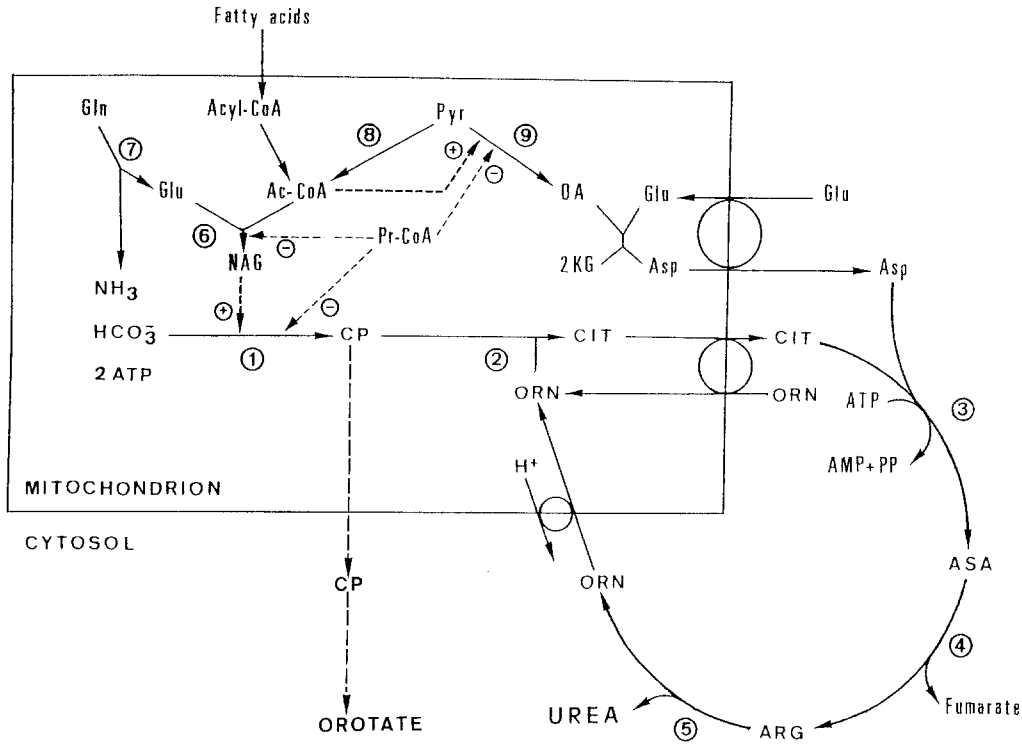

Fig. 1. Urea synthesis in liver tissue and its regulation. *Ac-CoA* acetyl-CoA; *Pr-CoA* propionyl-CoA; *Pyr* pyruvate, *OA* oxaloacetate, *2KG* 2-ketoglutarate; *NAG* N-acetylglutamate, *CP* carbamoylphosphate; *ASA* argininosuccinic acid. Enzymes = ① carbamoylphosphate synthetase I; ② ornithine transcarbamoylase; ③ argininosuccinate synthetase; ④ argininosuccinate lyase; ⑤ arginase; ⑥ N-acetylglutamate synthase; ⑦ glutaminase; ⑧ pyruvate dehydrogenase; ⑨ pyruvate carboxylase

direct measurement of citrulline by the diacetylmonoxime method, normally used for urea measurement (Ceriotti and Spandrio, 1965). The aim of this review is to show the complex metabolism of this amino acid, its physiological and pathological variations and to explain the mechanism of these variations.

Citrulline metabolism

Figure 2 shows tissular distribution of citrulline metabolism in which three organs (liver, small intestine and kidney) play different functions. In the liver citrulline is a metabolic intermediate involved in the elimination of one toxic compound (ammonia) through another non-toxic one (urea). The small intestine uses glutamine to produce almost all the circulating citrulline which is then used in kidneys to synthesize arginine. Other tissues such as brain, leukocytes and lymphocytes can also produce arginine from citrulline.

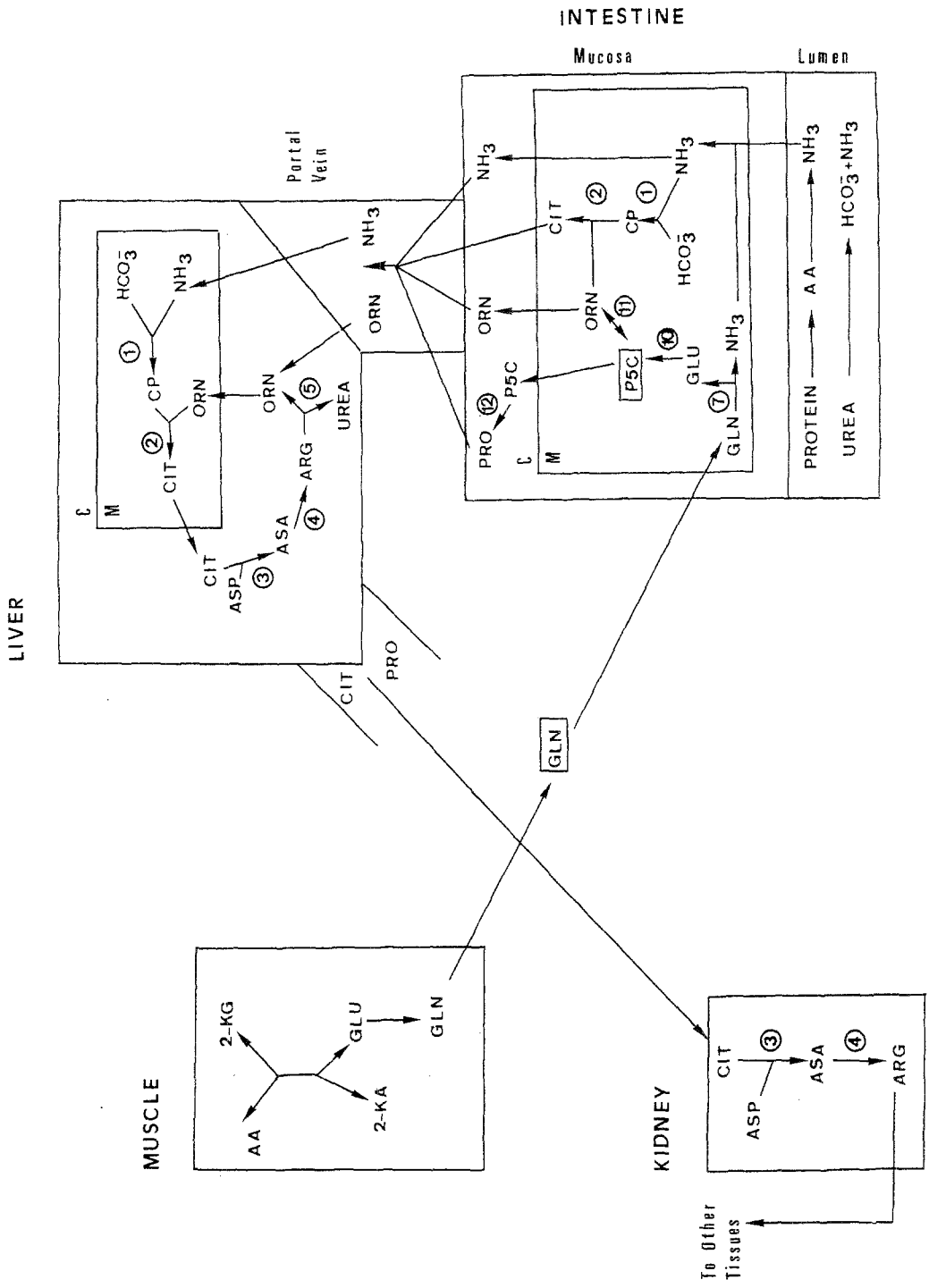

Fig. 2. Interorgan relationship for glutamine metabolism, ureagenesis and citrulline-arginine synthesis. Abbreviations not defined in Fig. 1: *P5C* Δ1-pyrroline-5-carboxylate; *AA* amino acids; *2KA* 2-ketoacids; *C* cytosol; *M* mitochondrion. Enzymes: ① to ⑨ similar to Fig. 1; ⑩ Δ1-pyrroline-5-carboxylate synthetase; ⑪ ornithine-δ-aminotransferase, ⑫ Δ1-pyrroline-5-carboxylate reductase

The biosynthesis of citrulline

The enzymes

Citrulline is synthesized in the mitochondria of the hepatocytes and the enterocytes (Fig. 1). Three enzymes control this synthesis: N-acetylglutamate synthase (NAGS, EC 2.3.1.1), carbamoylphosphate synthetase I (CPS-I, EC 6.3.4.16) and ornithine transcarbamoylase. In mitochondria, one molecule of ammonia is condensed to one molecule of bicarbonate to give carbamoylphosphate. This first reaction, driven by ATP, is catalysed by CPS-I, an enzyme localized in the mitochondrial matrix of hepatocytes and enterocytes (Gamble and Lehninger, 1973; Clarke, 1976; Raijman and Jones, 1976; Knecht et al., 1979). The nitrogen substrate is restricted to NH_3. Glutamine is not used as a substrate. CPS-I needs an obligate and specific activator, N-acetylglutamate (NAG) (Grisolia and Cohen 1953; Hall et al., 1958). This cofactor is synthesized in the mitochondrial matrix by the N-acetylglutamate synthase (Shigesada and Tatibana, 1971a, 1971b, 1978; Sonoda and Tatibana, 1983), an allosteric enzyme using L-arginine as an activator. This enzyme seems to be localized only in hepatocytes and enterocytes (Shigesada and Tatibana, 1971b). The second reaction, forming citrulline, is catalysed by ornithine transcarbamoylase, an enzyme also present in the mitochondrial matrix (Gamble and Lehninger, 1973) of hepatocytes (Jones et al., 1961) and enterocytes (Jones et al., 1961; Raijman, 1974; Hall et al., 1960; Herzfeld and Raper, 1976). The specific activity of OTC is about 40 to 50 times higher than that of CPS-I. Citrulline synthesis is also controlled by the mitochondrial transport systems for ornithine (Gamble and Lehninger, 1973; Chappel et al., 1972; Bradford and Mc Givan, 1980) the second substrate of OTC and those for NAG (Meijer et al., 1982) and arginine (Freedland et al. 1984), the latter being the activator of NAG synthase.

Other pathways of biosynthesis

Citrulline biosynthesis can be performed directly from arginine. This reaction, catalysed by a group of enzymes called NO-synthases, produces nitric oxide (NO) (Moncada, 1992):

$$\text{L-arginine} + O_2 \rightarrow \text{L-citrulline} + \text{NO}$$

The NO-synthase isoenzymes are located in the cytosol and need two cofactors: NADPH and tetrahydrobiopterine. They are specifically inhibited by a methylated derivative of arginine, the N-monomethyl-L-arginine. First identified in macrophages, (Hibbs et al., 1987; Iyengar R. et al., 1987), these NO-synthases were later found in endothelial cells. The nitric oxide formed in endothelial cells has relaxing properties on these cells and so is called "endothelium-derived relaxing factor" or "EDRF-nitric oxide". It has an activating effect on guanylate cyclase of target cells. In man, urinary excretion of nitrate may be a good indication of the amount of

citrulline synthesized by this pathway. In kidneys, citrulline can also be produced from dimethylarginine in another pathway, catalysed by the N^G,N^G-dimethylarginine dimethylaminohydrolase (Ogawa et al., 1989).

Utilization of citrulline

Citrulline leaves the mitochondrial matrix of hepatocytes and enterocytes by a transport system which allows its equimolar exchange with ornithine (Chappel et al., 1972; Bradford and Mc Givan, 1980). In the cytosol, citrulline is condensed to aspartate to give argininosuccinate, a precursor of arginine and urea. Three enzymes are involved in the transformation of citrulline into urea and ornithine: argininosuccinate synthetase (ASS, EC 6.3.4.5), argininosuccinate lyase (ASL, EC 3.5.3.1) and arginase (EC 3.5.3.1). Cytosolic concentration of aspartate is regulated by the activity of mitochondrial pyruvate carboxylase (PC, EC 6.4.1.1) which synthesizes oxaloacetate, co-substrate of the aspartate aminotransferase (EC 2.6.1.1). Pyruvate carboxylase is an allosteric enzyme, which uses acetyl-CoA as an activator. As NAG synthase activity, pyruvate carboxylase activity is dependent on fatty acid β-oxidation, pyruvate dehydrogenase activity and the presence of acyl-CoAs (propionyl-CoA, methylmalonyl-CoA) (Scrutton, 1974) which compete with acetyl-CoA.

Regulation of citrulline metabolism – The role of different tissues

Citrulline is an intermediate formed during urea synthesis in the liver

Liver is the only tissue containing the six enzymes which allow urea synthesis from ammonia. In these conditions citrulline produced in liver mitochondria is immediately transformed into arginine and urea. Liver is not able to export citrulline. Short-term regulation of liver citrulline synthesis depends on two factors: CPS-I activity and availability of ornithine (Meijer, 1979; Meijer and Hensgens, 1982; Mc Givan et al., 1976). Intramitochondrial activity of CPS-I seems to be regulated by the concentration of two of its substrates, ATP and ammonia (as NH_3) and its allosteric activator (NAG) and by the presence of some acyl-CoAs (propionyl-CoA, methylmalonyl-CoA). ATP is a key factor in the short-term regulation of this step because: (a) its concentration is controlled by the activity of the respiratory chain, (b) it is the substrate of other reactions (pyruvate carboxylase, argininosuccinate synthetase) in competition with CPS-I, (c) its hydrolysis gives ADP and inorganic phosphate, two inhibitors of CPS-I (Elliot and Tipton, 1974b), (d) N-acetylglutamate binding on CPS-I is dependent on ATP concentration (Elliot and Tipton, 1974a). The mitochondrial concentration of ammonia, the only nitrogen substrate of CPS-I, is limiting in physiological conditions. In normal physiological conditions ammmonia concentration is of the same order (0.3 – 0.7 mM) in portal blood (Mc Dermot, 1957; Warren, 1959; Brosnan, 1976) and in liver cells (Brosnan, 1976). However a large part of the intracellular ammo-

nia seems metabolically inert (Sainsbury, 1980; Wanders et al., 1980) and efficient NH_3 intramitochondrial concentration is lower than the Km value for CPS-I. For this reason, the reaction catalysed by CPS-I, should be the limiting step of citrulline synthesis. N-acetylglutamate, the allosteric activator of CPS-I, is also a key factor in the regulation of citrulline synthesis (Elliot and Tipton, 1974a; Grisolia and Cohen, 1953). For a given ammonia concentration the synthesis rate of carbamoylphosphate depends on NAG concentration. Several studies on whole animal (Tatibana and Shigesada, 1976; Saheki et al., 1977; Shigesada et al., 1978; Saheki et al., 1978; Stewart and Walser, 1980) or on isolated hepatocytes (Zollner, 1981; Hensgens et al., 1980; Aoyagi et al., 1979; Martin-Requero et al., 1983) have showed that citrulline (and urea) synthesis is correlated to the intracellular amount of NAG. Under these experimental conditions it is not possible to define exactly the role of NAG in the regulation of citrulline (or urea) synthesis. Indeed the concentration of some other metabolites such as ornithine, aspartate or arginine (Saheki et al., 1977; Stewart and Walser, 1980; Saheki et al., 1978) changes in the same way and may also influence the synthesis of citrulline (and urea). When isolated mitochondria were incubated with high concentration (10 mM) of NH_4Cl and ornithine, citrulline synthesis was tightly correlated to mitochondrial NAG content (Hensgens et al., 1980; Rabier et al., 1982). NAG synthesis is controlled at two levels: through the intramitochondrial concentration of the substrates (L-glutamate and acetyl-CoA), (Martin-Requero, 1983; Rabier et al., 1986) of activator (L-arginine) or of inhibitors (acyl-CoAs) of the NAG synthase or through the amount of the enzyme itself. Several workers (Krebs et al., 1973; Raijman and Jones 1976; Cohen et al., 1980) have suggested that ornithine has a direct effect on the regulation of CPS-I. In fact ornithine regulates citrulline synthesis via the modulation of intramitochondrial OTC activity. Ornithine supply to liver mitochondria is realized by cytosolic arginase which transforms arginine, produced either by metabolic pathways (urea cycle, proteolysis) or by diet, into ornithine which is then transported to the matrix by membrane transport systems (Gamble and Lehninger, 1973; Chappel et al., 1972; Bradford and Mc Givan 1980). De novo synthesis of ornithine does not seem to exist in liver mitochondria although a synthesis was reported in isolated hepatocytes (Lund and Wiggins, 1986). In some conditions, such as starvation, it is likely that ornithine is required for citrulline synthesis or for urea cycle activity and is supplied by the de novo synthesis from glutamine, located in the intestine (Windmueller, 1982). Citrulline produced in liver mitochondria is immediately exported to the cytosol where it reacts with aspartate to give argininosuccinate. Utilization of citrulline in liver is regulated at the level of the reaction catalysed by argininosuccinate synthetase. The main regulating factor of this reaction is aspartate which is synthesized in the mitochondria from pyruvate and glutamate via the two following reactions:

$$\text{Pyruvate} + HCO_3^- \rightarrow \text{Oxaloacetate} + \text{ATP (pyruvate carboxylase)}$$

$$\text{Oxaloacetate} + \text{glutamate} \underset{\leftarrow}{\longrightarrow} \text{Aspartate} + \alpha - \text{ketoglutarate}$$

(glutamate : oxaloacetate transaminase)

The regulating reaction in the aspartate biosynthesis is catalysed by pyruvate carboxylase, an allosteric enzyme, whose activator is acetyl-CoA.

Citrulline is an intermediate of the de novo biosynthesis of arginine. Role of the small intestine and the kidneys

Small intestine and kidneys are the only organs able to perform the synthesis of arginine from glutamine. They are complementary in this synthesis: the small intestine converts glutamine into citrulline which is transported to the kidneys to be transformed into arginine (Windmueller and Spaeth, 1981). In intestinal mucosa citrulline is one of the final products of glutamine metabolism. Citrulline is synthesized from ammonia produced by the deamidation of glutamine catalysed by the phosphate-dependent glutaminase (EC 3.5.1.2) (Windmueller, 1982; Pinkus and Windmueller, 1977) and from ornithine synthesized in situ from glutamine. As in liver mitochondria intestinal synthesis of citrulline is performed via the three steps catalysed by NAG synthase, CPS-I and OTC. In the intestine citrulline synthesis is not dependent on ornithine transport through the mitochondrial membrane as in liver since this amino acid is synthesized in the mitochondrial matrix from glutamine. Glutamine is either extracted from the bloodstream (Marliss et al., 1971; Felig, 1975) or supplied by protein hydrolysis in the intestinal lumen. About 10% of the carbon backbone of extracted glutamine is transformed to citrulline and ornithine and 30% of the α-amino nitrogen is in citrulline (Windmueller and Spaeth, 1974, 1975, 1978, 1980). Ornithine used for citrulline synthesis is formed from glutamine via glutaminase, Δ^1-pyrroline-5-carboxylate (P5C) synthase and ornithine aminotranferase (EC 2.6.1.13) (Fig. 2). P5C synthase activity is significantly expressed in only two tissues, intestinal mucosa and thymus (Jones, 1985; Wakabayashi, 1991). Since ASS and ASL activities are very low in enterocytes of adult rats (Ratner et Murakami, 1980; Ratner, 1983), citrulline produced by these cells can not be used in situ and is exported to be transformed to arginine. However in very young animals ASS and ASL have high specific activities up to weaning (Hurwitz and Kretchner, 1986) allowing the intestine to transform a large part of citrulline to arginine. Citrulline released by the small intestine in portal blood is not taken up by the liver but quantitatively removed by the kidneys (Windmueller and Spaeth, 1981; Dhanakoti et al., 1990; Tizianello et al., 1980) where citrulline is then transformed into arginine through the two reactions catalysed by ASS and ASL (Ratner and Murakami, 1980; Ratner, 1983). This transformation is regulated at two levels: (a) as in liver by ASS activity via aspartate availability, (b) by citrulline uptake in kidneys (Dhanakoti et al., 1990). Kidney seems to be the only organ able in physiological conditions to transform quantitatively citrulline to arginine (Windmueller and Spaeth, 1981). Other tissues, such as brain, lymphocytes, macrophages, (with ASS and ASL activities), do not seem to be able to significantly take up the citrulline released by the intestine. In these organs ASS and ASL activities seem to be used to regenerate in situ arginine transformed in nitric oxide and citrulline by NO-synthase activities.

Physiological and pathological variations of citrulline metabolism in man

In normal physiological conditions, liver does not take up or release citrulline. Therefore, the measurement of plasma concentration of this amino acid should represent the difference between the production by the intestine and the transformation into arginine by the kidneys.

Physiological variations

Citrulline is found in blood, urine, cerebrospinal fluid and amniotic fluid. In blood (drawn after one night starvation) plasma concentration is 26 ± 7 μmoles/l whatever the age of subjects (Kamoun et al., 1991). However this concentration is significantly lower during the first days of life (Kamoun et al., 1991; Bremer et al., 1981). It is likely that this observation is related to the presence of ASS and ASL activities in enterocytes as reported for newborns rats (Hurwitz and Kretchner, 1986). Plasma citrulline is poorly modified by meals. After a high-protein meal (1 g/kg body weight) its concentration slightly decreases and returns to normal value four hours later (Fig. 3). Urinary citrulline excretion is very low and shows very little variations with age (Kamoun et al., 1991; Bremer et al., 1981). The citrulline excretion is between 0 to 5 and 0 to 10 μmoles/mmole creatinine for subjects aged more or less than one year respectively. Citrulline is also found in amniotic fluid from the 8th week of gestation. Its concentration, almost constant up to the 30th week, progressively decreases until birth (O'Neill et al., 1971; Reid et al., 1971; Kang and Scanlon, 1974). Early presence of citrulline in amniotic fluid is related to the early appearance (9th week) of the urea cycle enzymes in fetal liver (Räihä and Suihkonen, 1968; Guha and Mukherjee, 1974). In contrast the

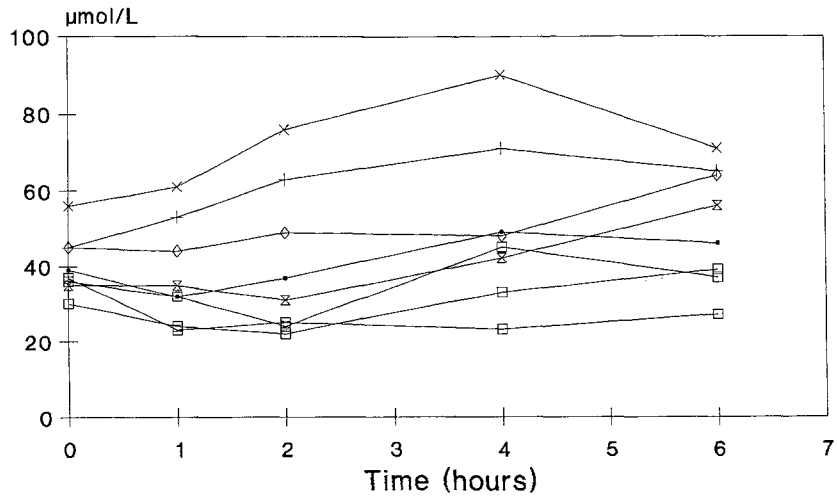

Fig. 3. Citrulline variation during protein loading test (1 g·kg^{-1} body weight) in heterozygotes for citrullinemia (□ controls: ×, +, ·, x, ◇ heterozygotes)

decrease observed at the end of pregnancy may reflect an increased need of arginine for protein synthesis by fetal tissues.

Pathological conditions with increased citrullinemia

Citrulline levels increase in all situations where its transformation to urea or arginine is impaired. These modifications are caused either by enzymatic deficiency (ASS, ASL, arginase or pyruvate carboxylase) or by chronic renal failure since the kidney is the main tissue using citrulline. Table 1 summarizes the causes of hypercitrullinemia and shows in each case the variations of the other amino acid concentrations in plasma and urine.

a) ASS deficiency (citrullinemia)

Citrullinemia results from the deficiency of ASS, the enzyme in the urea cycle which catalyses the first reaction for transformation of citrulline into arginine or urea. Three variants of citrullinemia exist, all exhibiting a hyperammonemia, and an important increase of citrulline. In types I (ASS with kinetics abnormalities) and III (no detectable ASS activity) abnormalities are expressed in all the tissues (liver, kidneys, fibroblasts) while in type II, where the deficiency is caused by a decreased amount of enzyme protein abnormalities are expressed in the liver but not in the kidneys and fibroblasts (Saheki et al., 1985, 1987). In neonatal patients, where residual activity is either very low or null (type I and III), plasma concentration of citrulline is very high (>1,500 μmoles/l) whereas it is lower (<1,000 μmoles/l) in patients with high residual activity in liver and normal activity in kidneys (Saheki et al., 1985, 1987). In Type I heterozygotes, with 50% of the normal ASS activity (Kenaway et al., 1975), plasma citrulline is more elevated than in controls: 49 ± 13 μmoles/l, in 10 obligate heterozygotes (unpublished results) (Clemens and Plettner, 1989). In these heterozygotes plasma citrulline concentration significantly increases after an acute protein load (1 g/kg body weight), whatever the initial concentration (Fig. 3).

Liver transplantation proposed as a therapeutic means to correct the deleterious effects of hyperammonemia allows the restoration of normal ammonia detoxication but it has no effect on arginine synthesis because of the persistence of the enzymatic deficiency in kidneys. The elevated citrullinemia (200–600 μmoles/l) observed after liver transplantation, whatever the protein intake (Rabier et al., 1991), is exclusively from intestinal origin.

b) ASL deficiency (argininosuccinic aciduria)

ASL is the second cytosolic enzyme of the urea cycle and allows the transformation of citrulline into arginine. ASL deficiency results in an increase of citrulline in plasma (100–300 μmoles/l) and urine, but to a lesser extent than for ASS deficiency. The enzymatic defect also induces an accumulation of an

unusual amino acid, argininosuccinic acid, produced from the condensation of citrulline and aspartate. The plasma concentration of ammonia-dependent amino acids (glutamic acid, glutamine and alanine) and lysine are also increased. Liver and kidneys are the main organs responsible for citrulline accumulation.

c) Arginase deficiency (hyperargininemia)

In hyperargininemia, the hepatic isoform of arginase is deficient and citrullinemia is normal or slightly increased (Walser, 1983; Marescau et al., 1979). This may be explained by the lack of ornithine synthesis due to the arginase deficiency and the normal utilization of citrulline for arginine synthesis in kidneys.

d) Pyruvate carboxylase deficiency

Pyruvate carboxylase catalyses the carboxylation of pyruvate to oxaloacetate which is then transaminated to aspartate, a substrate of ASS. Pyruvate carboxylase deficiency causes a decrease of aspartate biosynthesis and in this way it induces an inhibiting effect on the ASS activity. Citrulline, the other substrate of ASS, therefore accumulates. The two variants (A and B) of pyruvate carboxylase deficiency are both characterized by a hyperlactacidemia (Robinson, 1989): only Type B, the neonatal onset form, discloses hyperammonemia and increased plasma citrulline concentration (50 to 300 μmol/l) associated with a peculiar chromatographic profile of plasma amino acids (Charpentier et al., 1982): glutamic acid and glutamine concentrations are decreased despite hyperammonemia whereas proline and lysine concentrations always increase (Table 1).

e) Lysinuric protein intolerance (LPI)

This disease results from an abnormality of the cellular efflux of dibasic amino acids (lysine, ornithine and arginine) (Simell, 1989). In epithelial cells of gut and kidney a specific transport system has been well characterized and localized on the basolateral membrane (Desjeux et al., 1980). In the other cells (fibroblasts, hepatocytes) such a system exists, but it exact localization is undefined, although it is suspectd to be in either plasma membrane or membrane of subcellular organelles. Patients with LPI have a hyperammonemia, an increased urinary excretion of orotic acid and a peculiar chromatographic profile of amino acids: in plasma there is an increase of glutamine and citrulline, together with a huge decrease of lysine, ornithine and arginine while in urine, citrulline, lysine, ornithine and arginine are highly increased and cystine moderately increased. The most likely explanation for the increase of citrulline in plasma and urine is the stimulated synthesis of citrulline in enterocytes by the increase of intracellular ornithine and arginine associ-

Table 1. Main causes of hypercitrullinemia

Citrulline		Other constant abnormalities		Possible abnormalities		Ammonemia	Orotic acid	Enzyme deficiency or dysfunction
Plasma (μM) 400–4500 (Type I et III) 100–1500 (Type II)	Urine ↗ ↗	Plasma ↘Arg ↘(Orn)	Urine –	Plasma ↗Glu+Gln ↗Pro ↗Ala ↗Lys	Urine	↗	↗	Argininosuccinate synthetase deficiency
125–440 ↘	↗	↗ASA ↘Orn ↘Arg	 – –	Others AA N or ↗ ↗Glu+Gln ↗Pro ↗Ala ↗Lys Others AA N or ↗		↗	↗	Argininosuccinate lyase deficiency
45–90 ↘	↗	 ↗Pro ↘Glu+Gln	↗Lys – –	–		↗	N	Pyruvate carboxylase deficiency
25 à 50 ↘	N or ↗	↗Arg	–	–	↗Orn ↗Lys ↗Cys	N or ↗	↗	Arginase deficiency
30–60 ↘	↗	↘Lys ↘Orn ↘Arg	↗Lys ↗(Hci)	↗Glu+Gln	 ↗Orn ↗Arg ↗Cys	↗	↗	 Lysinuric protein intolerance
G1 = 60 ± 21* G2 = 76 ± 21 G3 = 91 ± 50	N or ↗	↗Cys ↗(3 MeHis) ↗(Orn)	↗(Cys) ↗(Lys)	↗Pro ↗Tau ↗Gly ↘BCAA	–	N	N	Renal failure
<5	N	↗Arg N or ↗Orn		↗Glu+Gln, ↗Pro, ↗Ala, ↗Lys, Other AA N or ↗		↗	↗ N N	*Neonatal onset* OTC deficiency CPS deficiency NAGS deficiency
10 à 25 ↘	N	–		↗(Glu+Gln), ↗(Ala), ↗(Lys), ↗(Pro), ↗(Arg)		N or ↗	↗ N N	*Late onset* OTC deficiency CPS deficiency NAGS deficiency
5 à 15 ↘	N	↘Pro ↘Orn	–			↗fasted N after meal	N	Pyrroline-5-carboxylate synthetase deficiency

↗ increase; ↘ decrease; *N* normal; () variable modifications; * G1, G2, G3 correspond to the groups as defined in Ceballos et al. (1990) Clin Chim Acta 188:101–108

ated with the inhibiting effect of the increased intracellular dibasic amino acids concentrations on the renal ASS and ASL resulting in the decrease of renal utilization of citrulline for the arginine synthesis.

f) Renal failure

Kidney is the main organ synthesizing arginine from citrulline, since ASS and ASL are located along its tubules (Dhanakoti et al., 1990; Levillain et al., 1990). Any destruction of the renal tissue will induce an impairment in the citrulline metabolism. In chronic renal failure, plasma citrulline concentration increases as well as cystine and 3-methylhistidine. Plasma citrulline concentrations increase very early and rise according to the progression of renal failure and are always well-correlated to the plasma creatinine (Ceballos et al., 1990). In experimental renal failure it was shown that the increase of plasma citrulline represented a compensating mechanism allowing the leaving nephrons to take up more citrulline per unit length in order to maintain a constant synthesis of arginine (Bouby et al., 1993).

g) Hepatocellular failure

In hepatic failure, intracellular amino acids flow into the bloodstream and contribute to a significant increase in amino acidemia. Plasma citrulline concentration is increased as well as other amino acids but its concentration is normal when expressed as a percentage of the total amino acidemia.

Pathological conditions with decreased citrullinemia

Plasma citrulline concentration is a result of the difference between intestinal synthesis and renal utilization. Any decrease of citrulline concentration will be related to a defect of intestinal synthesis. Impairment of synthesis results from either a molecular abnormality of one of the mitochondrial enzymes which control the intestinal synthesis of citrulline from glutamine (Fig. 2) or a destruction of intestinal mucosa, the main tissue which exports citrulline.

a) Deficiency of a mitochondrial urea cycle enzyme: NAG synthase, CPS-I or OTC

Because these three enzymes control the biosynthesis of carbamoylphosphate from ammonia and bicarbonate and its condensation with ornithine to give citrulline, the total or partial deficiency of one of these enzymes induces an impairment of the intestinal synthesis of citrulline. The same enzymes are also present in liver mitochondria, and a deficiency in the liver is essentially expressed by hyperammonemia. Impairment of intestinal biosynthesis of

citrulline will result in a lower supply of citrulline to the kidneys and a decrease of arginine biosynthesis.

Two types of clinical onsets should be distinguished: the neonatal onset (1 to 7 days of life) and the late onset (few weeks to adult). In the former patients residual enzyme activities are very low (<2%) or null (Walser, 1983; Briand et al., 1982). In these conditions citrulline synthesis is near zero and its plasma concentration is between 0 and 5 μmoles/l. In the latter patients, residual enzyme activities can be quite elevated (Walser, 1983; Briand et al., 1982), and plasma citrulline concentration is almost normal (Table 1). Sometimes a decrease can only be shown by using its relative concentration (expressed as a percentage of total amino acidemia). The plasma citrulline concentration is not specific enough to distinguish NAG synthase, CPS-I and OTC deficiencies. Urinary orotate excretion (basal or after protein or/and allopurinol loading tests) allows the diagnosis of OTC deficiency.

Liver transplantation is currently the only available form of enzyme – replacement therapy for patients with CPS-I and OTC deficiency offering protection from the deleterious effects of hyperammonemia – However hypocitrullinemia and hypoargininemia are always observed after liver transplantation (Tuchman, 1989; Largillière et al., 1989; Rabier et al., 1991) because the intestinal defect is unmodified. This observation confirms the special function of the small intestine and kidneys in the biosynthesis of arginine and the minor role of the liver in this metabolic pathway.

b) Δ^1 pyrroline 5-carboxylate synthase deficiency

Two children, brother and sister, both with cataract, joint hyperlaxity and severe mental retardation, were hospitalized in the metabolic unit of Necker – Enfants Malades Hospital because hyperammonemia was observed in the girl. This hyperammonemia was observed mainly after fasting and was paradoxically corrected by meals. Urinary orotic acid excretion was normal. However, we observed an abnormally low concentrations of proline, citrulline, ornithine and arginine. The persistence of these biochemical abnormalities leads us to suspect a deficiency of the Δ^1 pyrroline-5- carboxylate synthase (Fig. 2) that was confirmed by the measurement of the transformation of (U-^{14}C) glutamate in (U-^{14}C) proline in intact fibroblasts (unpublished results). On the other hand an oral load of ornithine to both patients induced a significant increase in plasma concentration of citrulline, arginine and proline while a glutamine load had no effect on the plasma concentration of these amino acids in contrast to the opposite observation made in normal subjects (Dechelotte et al., 1991). The deficiency of this intestinal enzyme activity will lead to the defect of ornithine synthesis. The first consequence is a decrease of the synthesis of citrulline in the intestine and of the synthesis of arginine in the kidneys. The second effect is a defective supply of ornithine to the liver during the fasting periods causing subsequent hyperammonemia (during feeding periods ornithine supply is sustained by dietary arginine).

c) Coeliac disease

Coeliac disease is characterized by a destruction of intestinal villosities. This atrophy is accompained by a decrease in the three mitochondrial enzymes of the urea cycle. For this reason the degree of hypocitrullinemia observed (Hernanz and Polanco, 1991) will be related to the degree of the intestinal atrophy. After treatment citrullinemia returns to normal values.

d) Miscellaneous

Plasma citrulline is decreased along with other amino acids in patients with glucagonome or by perfusion of glucose solute. However, when expressed as a percentage of total amino acidemia, plasma citrulline concentrations appear normal. We also observed a significant and specific decrease of plasma citrulline in some patients with a defect in one of the respiratory chain complexes. This abnormality was already reported for a patient with Pearson's syndrome (Ribes et al., 1993). This hypocitrullinemia can be explained either by an atrophy of intestinal villosities or by an energy defect which depletes the reactions catalysed either by P5C synthase or by CPS-I in their energetic substrate, ATP.

Conclusion

Citrulline is a non-protein amino acid involved in three different metabolic pathways: hepatic detoxication of ammonia into urea, de novo synthesis of arginine from glutamine and synthesis of nitric oxide. The interpretation of its physiopathological variations in plasma is tightly linked to the knowledge of these metabolic processes and their tissular location. The recent discovery of a new pathway for the synthesis of citrulline (by NO-synthases) gives a new interest in the study of citrulline metabolism.

References

Aoyagi K, Mori M, Tatibana M (1979) Inhibition of urea synthesis by pent-4-enoate associated with decrease in N-acetylglutamate concentration in isolated rat hepatocytes. Biochim Biophys Acta 587: 515–521

Bouby N, Hassler C, Parvy P, Bankir L (1993) Renal synthesis of arginine in chronic renal failure: in vivo and in vitro studies in rats with 5/6 nephrectomy. Kidney Int 49: 676–683

Bradford NM, Mc Givan JD (1980) Evidence for the existence of an ornithine/citrulline antiporter in rat liver mitochondria. FEBS Lett 113: 294–298

Bremer HJ, Duran M, Kamerling JP, Przyrembel H, Wadman SK (1981) Disturbances of amino acid metabolism: clinical chemistry and diagnosis. Urban and Schwarzenberg, Baltimore Munich, pp 203–227

Briand P, François B, Rabier D, Cathelineau L (1982) Ornithine transcarbamylase deficiencies in human males. Kinetic and immunochemical classification. Biochim Biophys Acta 704: 100–106

Brosnan JT (1976) Factor affecting intracellular ammonia concentration in liver. In: Grisolia S, Baguena R, Mayor F (eds) The urea cycle J. Wiley and Sons, New York, pp 443–457

Ceballos I, Chauveau P, Guerin V, Bardet J, Parvy P, Kamoun P, Jungers P (1990) Early alterations of plasma amino acids in chronic renal failure. Clin Chim Acta 188: 101–108

Ceriotti G, Spandrio L (1965) Catalytic acceleration of the urea-diacetylmonoxime-phenazone reaction and its application to automatic analysis. Clin Chim Acta 11: 519–522

Chappel JB, Mc Givan JD, Crompton M (1972) In: Woessner JF, Huising JF, Huising F (eds) The molecular basis of biological transport. Academic Press, New York, pp 55–81

Charpentier C, Tetau JM, Ogier H, Saudubray JM, Coudé FX, Lemmonier A (1982) Amino acid profile in pyruvate carboxylase deficiency: comparison with some other metabolic disorders. J Inherit Metab Dis 5[Suppl]1: 11–12

Clarke S (1976) A major polypeptide component of rat liver mitochondria: carbamyl-phosphate synthetase. J Biol Chem 251: 950–961

Clemens PC, Plettner Ch (1989) A non-enzymatic method for identification of citrullinemia heterozygotes. Clin Genet 35: 468–469

Cohen NS, Cheung CW, Raijman L (1980) The effects of ornithine on mitochondrial carbamylphosphate synthesis. J Biol Chem 255: 10248–10255

Dechelotte P, Darmaun D, Rongier M, Hecketsweiler B, Rigal O, Desjeux JF (1991) Absorption and metabolic effects of enterally administered glutamine in humans. Am J Physiol 260: G677–G682

Desjeux JF, Rajantie J, Simell O, Dumontier AM, Perheentupa J (1980) Lysine fluxes across the jejunal epithelium in lysinuric protein intolerance. J Clin Invest 65: 1382–1387

Dhanakoti SN, Brosnan JT, Herzberg GR, Brosnan ME (1990) Renal arginine synthesis: studies in vitro and in vivo. Am J Physiol 259: E437–E442

Elliot KRF, Tipton KF (1974a) Kinetic studies of bovine liver carbamoylphosphate synthase. Biochem J 141: 807–816

Elliot KRF, Tipton KF (1974b) Product inhibition studies on bovine liver carbamoyl-phosphate synthetase. Biochem J 141: 817–824

Felig P (1975) Amino acid metabolism in man. Ann Rev Biochem 44: 933–955

Freedland RA, Crozier GL, Hicks BL, Meijer AJ (1984) Arginine uptake by isolated rat liver mitochondria. Biochim Biophys Acta 802: 407–412

Gamble JG, Lehninger AL (1973) Transport of ornithine and citrulline across the mitochondrial membrane. J Biol Chem 248: 610–618

Grisolia S, Cohen PP (1953) Catalytic role of glutamate derivatives in citrulline synthesis. J Biol Chem 204: 753–757

Guha SK, Mukherjee KL (1974) Urea biosynthesis in normal human fetuses. Biochim Biophys Acta 372: 285–290

Hall LM, Metzenberg RL, Cohen PP (1958) Isolation and characterization of a naturally occuring cofactor of carbamylphosphate synthesis. J Biol Chem 230: 1013–1021

Hall LM, Johnson RC, Cohen PP (1960) The presence of carbamoylphosphate synthetase in intestinal mucosa. Biochim Biophys Acta 37: 144–145

Hensgens HESJ, Verhoeven AJ, Meijer AJ (1980) The relationship between intramitochondrial N-acetylglutamate and activity of carbamoylphosphate synthetase (ammonia). The effect of glucagon. Eur J Biochem 107: 197–205

Hernanz A, Polanco I (1991) Plasma precursor aminoacids of central nervous system monoamines in children with coeliac disease. Gut 32: 1478–1481

Herzfeld A, Raper SM (1976) Enzymes of ornithine metabolism in adult and developing rat intestine. Biochim Biophys Acta 428: 600–610

Hibbs JB, Taintor RR, Vavrin Z (1987) Macrophage cytotoxicity: Role for L-arginine deiminase and imino nitrogen oxidation to nitrate. Science 235: 473–476
Hurwitz R, Kretchner N (1986) Development of arginine synthesizing enzymes in mouse intestine. Am J Physiol 251: G103–G110
Iyengar R, Stuehr DJ, Marletta MA (1987) Macrophage synthesis of nitrite, nitrate and N-nitrosamines: Precursors and role of the respiratory burst. Proc Natl Acad Sci USA 84: 6369–6373
Jones ME (1985) Conversion of glutamate to ornithine and proline: pyrroline-5-carboxylate, a possible modulator of arginine requirements. J Nutr 115: 509–515
Jones ME, Anderson AD, Anderson C, Hodes S (1961) Citrulline synthesis in rat tissues. Arch Biochem Biophys 95: 499–507
Kamoun P, Parvy P, Cathelineau L (1983) A rapid and simple method for citrulline determination in plasma. Clin Chem 29: 398–400
Kamoun P, Parvy Ph, Rabier D (1991) Indications et interprétation de la chromatographie des acides aminés pour le diagnostic des maladies métaboliques. In: Saudubray JM (ed) Maladies métaboliques. Doin, Paris, pp 1–13
Kang ES, Scanlon J (1974) Concentrations of the free amino acids in human amniotic fluid during normal and abnormal pregnancies. Am J Obstet Gynecol 119: 603–609
Kennaway NG, Harwood PJ, Ramberg DA, Koler RD, Buist NRM (1975) Citrullinemia: enzymatic evidence for genetic heterogeneity. Pediatr Res 9: 554–558
Knecht E, Hernandez J, Wallace R, Grisolia S (1979) Immunoferritin location of carbamoylphosphate synthetase. J Histochem Cytochem 27: 975–981
Krebs HA, Hems R, Lund P (1973) Some regulatory mechanism in the synthesis of urea in the mammalian liver. Adv Enzyme Regul 11: 361–377
Largillière C, Houssin D, Gottrand F, Mathey C, Checoury A, Alagille D, Farriaux JP (1989) Liver transplantation for ornithine transcarbamylase deficiency in a girl. J Pediatr 115: 415–417
Levillain D, Hus-Citharel A, Morel F, Bankir L (1990) Localization of arginine synthesis along rat nephron. Am J Physiol 259: F916–F923
Lund P, Wiggins D (1986) The ornithine requirement of urea synthesis. Formation of ornithine from glutamine in hepatocytes. Biochem J 239: 773–776
Marescau B, Pintens J, Lowenthal A, Terheggen HG, Adriaenssens K (1979) Arginase and free amino acids in hyperargininemia. Leukocyte arginine as a diagnostic parameter for heterozygotes. J Clin Chem Clin Biochem 17: 211–217
Marliss EB, Aoki TT, Pozefsky T, Most AS, Cahill GF (1971) Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. J Clin Invest 50: 814–817
Martin-Requero A, Corkey BE, Cerdan S, Walajtys-Rode E, Parilla RL, Williamson JR (1983) Interactions between α-ketoisovalerate metabolism and the pathways of gluconeogenesis and urea synthesis in isolated hepatocytes. J Biol Chem 258: 3673–3681
Mc Dermot WV (1957) Metabolism and toxicity of ammonia. N Engl J Med 257: 1076–1081
Mc Givan JD, Bradford NM, Mendes-Mourao J (1976) The regulation of carbamoylphosphate synthase activity in rat liver mitochondria. Biochem J 154: 415–421
Meijer AJ (1979) Regulation of carbamoylphosphate synthetase (ammonia) in liver in relation to urea cycle activity. Trend Biochem Sci 4: 83–86
Meijer AJ, Hensgens HESJ (1982) Ureogenesis. In: Sies H (ed) Metabolic compartmentation. Academic Press, New York, pp 817–824
Meijer AJ, Van Waoerkom GM, Wanders RKJA, Lof C (1982) Transport of N-acetylglutamate in rat liver mitochondria. Eur J Biochem 124: 325–330
Moncada S (1992) The L-arginine: nitric oxide pathway. Acta Physiol Scand 145: 201–227
O'Neil R, Morrow G, Hammel D, Auerbach VH, Barness LA (1971) Diagnostic significance of amniotic fluid amino acids. Obstet Gynecol 37: 550–554

Ogawa T, Kimoto M, Sasaoka K (1989) Purification and properties of a new enzyme, ^{NG,NG}-dimethylarginine dimethylaminohydrolase, from rat kidney. J Biol Chem 264: 10205–10209

Pinkus LM, Windmueller HG (1977) Phosphate-dependent glutaminase of small intestine: localization and role in intestinal glutamine metabolism. Arch Biochem Biophys 182: 506–517

Rabier D, Briand P, Petit F, Kamoun P, Cathelineau L (1986) Effects of organic acids on the synthesis of citrulline by intact rat liver mitochondria. Biochimie 68: 639–647

Rabier D, Briand P, Petit F, Parvy P, Kamoun P, Cathelineau L (1982) Acute effects of glucagon on citrulline biosynthesis. Biochem J 206: 627–631

Rabier D, Narcy C, Bardet J, Parvy Ph, Saudubray JM, Kamoun P (1991) Arginine remains an essential amino acid after liver transplantation in urea cycle enzyme deficiencies. J Inherit Metab Dis 14: 277–280

Räihä NCR, Suihkonen J (1968) Development of urea-synthesizing enzymes in human liver. Acta Paediatr Scand 57: 121–127

Raijman L (1974) Citrulline synthesis in rat tissues and liver content of carbamoylphosphate and ornithine. Biochem J 138: 225–232

Raijman L, Jones ME (1976) Purification, composition and some properties of rat liver carbamylphosphate synthetase (ammonia). Arch Biochem Biophys 175: 270–278

Ratner S (1983) A radiochemical assay for argininosuccinate synthetase with [U-^{14}C] aspartate. Anal Biochem 135: 479–488

Ratner S, Murakami-Murofuski K (1980) A new radiochemical assay of arginosuccinase with purified [^{14}C] argininosuccinate. Anal Biochem 106: 134–147

Reid DW, Campbell DJ, Yakymyshyn LY (1971) Quantitative amino acids in amniotic fluid and maternal plasma in early and late pregnancy. Am J Obstet Gynecol 111: 251–258

Ribes A, Riudor E, Valcarel R, Salva A, Castello F, Murillo S, Dominguez C, Rotig A, Jakobs C (1993) Pearson syndrome: altered tricarboxylic acid and urea-cycle metabolites, adrenal insufficiency and corneal opacities. J Inherit Metab Dis 16: 537–540

Robinson BH (1989) Lactic acidemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease. Mac Graw Hill, New York, pp 869–888

Saheki T, Katsunuma T, Sase M (1977) Regulation of urea synthesis in rat liver. Changes of ornithine and acetylglutamate concentrations in the livers of the rats subjected to dietary transitions. J Biochem 82: 551–558

Saheki T, Ohkubo T, Katsunuma T (1978) Regulation of urea synthesis in rat liver. Increase in the concentrations of ornithine and acetylglutamate in rat liver in response to urea synthesis stimulated by the injection of an ammonium salt. J Biochem 84: 1423–1430

Saheki T, Nakano K, Kobayashi K, Imamura Y, Itakura Y, Sase M, Hagihara S, Matuo S (1985) Analysis of the enzyme abnormality in eight cases of neonatal and infantile citrullinaemia in Japan. J Inherit Metab Dis 8: 155–156

Saheki T, Kobayashi K, Inoue I, Matuo S, Haihara S, Noda T (1987) Increased urinary excretion of argininosuccinate in type II citrullinemia. Clin Chim Acta 170: 297–304

Sainsbury GM (1980) The distribution of ammonia between hepatocytes and extracellular fluid. Biochim Biophys Acta 631: 305–316

Scrutton MC (1974) Pyruvate carboxylase. Studies of activator-independent catalysis and of the specificity of activation by acyl derivatives of coenzyme A for the enzyme from rat liver. J Biol Chem 249: 7057–7067

Shigesada K, Aoyagi K, Tatibana M (1978) Role of acetylglutamate in ureotelism. Variation of acetylglutamate level and its possible significance in control of urea synthesis in mammalian liver. Eur J Biochem 85: 385–391

Shigesada K, Tatibana M (1971a) Enzymatic synthesis of acetylglutamate by mammalian liver preparations and its stimulation by arginine. Biochem Biophys Res Commun 44: 1117–1124

Shigesada K, Tatibana M (1971b) Role of actylglutamate in ureotelism. I. Occurence and biosynthesis of actylglutamate in mouse and rat tissues. J Biol Chem 246: 5588–5595

Shigesada M, Tatibana M (1978) N-acetylglutamate synthetase from rat liver mitochondria. Partial purification and catalytic properties. Eur J Biochem 84: 285–291

Simell O (1989) Lysinuric protein intolerance and other cationic aminoacidurias. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease. Mac Graw Hill, New York, pp 2497–2513

Sonoda T, Tatibana M (1983) Purification of N-acetylglutamate synthetase from rat liver mitochondria and substrate and activator specificity of the enzyme. J Biol Chem 258: 9839–9844

Stewart PM, Walser M (1980) Short term regulation of ureogenesis. J Biol Chem 255: 5270–5280

Tatibana M, Shigesada K (1976) Regulation of urea biosynthesis by the acetylglutamate-arginine system. In: Grisolia S, Baguena R, Mayor F (eds) The urea cycle. J Wiley and Sons, New York, pp 301–313

Tizianello A, De Ferrari G, Garibotto G, Gurreri G, Robaudo C (1980) Renal metabolism of amino acids and ammonia in subjects with normal renal function and in patients with chronic renal insufficiency. J Clin Invest 65: 1162–1173

Tuchman M (1989) Persistant acitrullinemia after liver transplantation for carbamylphosphate synthetase deficiency. N Engl J Med 320: 1488–1499

Wakabayashi Y, Yamada E, Hasegawa T, Yamada RN (1991) Enzymological evidence for the indispensability of small intestine in the synthesis of arginine from glutamate. Arch Biochem Biophys 291: 1–8

Walser M (1983) Urea cycle disorders and other hereditary hyperammonemic syndromes. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, Goldstein JL, Brown MS (eds) The metabolic basis of inherited disease. Mac Graw Hill, New York, pp 402–438

Wanders RJA, Hoek JB, Tager JM (1980) Origin of the ammonia found in protein-free extracts of rat liver mitochondria and rat hepatocytes. Eur J Biochem 110: 197–202

Warren JT (1959) Portal and peripheral blood ammonia concentration in germ-free and conventional guinea pigs. Am J Physiol 197: 717–720

Windmueller HG (1982) Glutamine utilization by the small intestine. Adv Enzymol 53: 201–237

Windmueller HG, Spaeth AE (1974) Uptake and metabolism of plasma glutamine by the small intestine. J Biol Chem 249: 5070–5079

Windmueller HG, Spaeth AE (1975) Intestinal metabolism of glutamine and glutamate from the lumen as compared to glutamine from blood. Arch Biochem Biophys 171: 662–672

Windmueller HG, Spaeth AE (1978) Identification of ketone bodies and glutamine as the respiratory fuels in vivo for postabsorptive rat small intestine. J Biol Chem 253: 69–76

Windmueller HG, Spaeth AE (1980) Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. Quantitative importance of glutamine, glutamate and aspartate. J Biol Chem 255: 107–112

Windmueller HG, Spaeth AE (1981) Source and fate of circulating citrulline. Am J Physiol 241: E473–E480

Zollner H (1981) Regulation of urea synthesis. The effect of ammonia on the N-acetylglutamate content of isolated rat liver. Biochim Biophys Acta 676: 170–176

Authors' address: Dr. D Rabier, Laboratoire Biochimie Médicale B-URA CNRS 1335, Hôpital Necker – Enfants Malades, 149, rue de Sèvres, F-75743 Paris Cedex 15, France.

Received May 4, 1995