Urea biosynthesis I.
The urea cycle and relationships to the citric acid cycle1, 2

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ABSTRACT  The urea cycle consist of five enzymatically controlled steps that are catalyzed by carbamyl phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinase, and arginase, respectively. The complete cycle is present in physiological meaningful levels in the liver of terrestrial vertebrates, and in man represents the sole mechanism for ammonia disposal. The formation of carbamyl phosphate and the synthesis of argininosuccinate are potential limiting steps in urea biosynthesis but substrate and not enzymes levels are rate-limiting under physiological conditions. In the adult, urea cycle enzymes change as a unit, and are largely influenced by dietary protein content. The urea cycle is closely linked to the citric acid cycle deriving one of its nitrogens through transamination of oxalacetate to form aspartate and returns fumarate to that cycle. The biosynthesis of urea demands the expenditure of energy but less than 20% of the energy derived from metabolism of gluconeogenic amino acids is required for ureogenesis. Embryological development of the urea cycle in the tadpole and in mammalian fetal liver therefore permits use of amino acids as new sources of energy to meet oxidative demands for continuing growth.  Am. J. Clin. Nutr. 30: 2083-2087, 1977.

Urea biosynthesis developed as a survival mechanism when primitive animals made the transition from Devonian seas to a swampy terrestrial existence. Ammonia generated via cell metabolism in the fish can easily diffuse into the surrounding water through the gills, but accumulation of this toxic product in the air-breathing amphibian progenitor necessitated the conversion of ammonia to a nontoxic metabolic end product. The mechanism of urea formation from NH3 and CO2 was proposed in 1932 by Krebs and Hensleit (1) to be a cyclic process wherein ornithine, citrulline and arginine acted as carrier compounds. The recapitulation of phylogeny in the metamorphosing tadpole that begins life as an amnonotole larva and gradually becomes a ureotelic frog has provided a model for assessing specific biochemical systems required for the transition from ammonotelism to ureotelism (2-4). The proposed ornithine cycle has now been clarified in its entirety (5), and today is understood to consist of five enzymatically controlled steps: 1) conversion of bicarbonate and ammonia to carbamyl phosphate; 2) formation of citrulline from ornithine and carbamyl phosphate; 3) conversion of citrulline and aspartate to argininosuccinate; 4) cleavage of argininosuccinate to form arginine; 5) degradation of arginine to form urea and ornithine. The ornithine so formed is now available for reutilization in step 2), and the urea is excreted.

These steps are catalyzed by two mito-

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chondrial enzymes, carbamyl phosphate synthetase and ornithine transcarbamylase, and by three cytoplasmic enzymes, argininosuccinate synthetase, argininosuccinase, and arginase.

All five enzymes are present in the liver, gut, kidney, but only in the liver are they present in levels capable of supporting physiologically meaningful ureogenesis (6). The component parts of the urea cycle in terrestrial vertebrates i.e., amphibians and mammals, are closely related to the citric acid cycle (Fig. 1). In this figure, the urea cycle is shown on the left and the citric acid cycle on the right. The numbers refer to the five steps involved in the biosynthesis of urea, and asterisks refer to the amino nitrogen that is destined to form urea. The cycle starts at the upper left where the initial step is the conversion of CO\(_2\) and NH\(_3\) to carbamyl phosphate, a reaction catalyzed by the enzyme carbamyl phosphate synthetase (CPS I). A necessary cofactor for this reaction in N-acetyl glutamate (NAG) and the sequence is postulated to be (5):

(a) \[\text{CPS} \cdot \text{NAG} + \text{CO}_2 + \text{ATP} \rightarrow \text{CPS} \cdot \text{NAG} \cdot \text{CO}_2 \sim \text{P} + \text{ADP}\]

(b) \[\text{CPS} \cdot \text{NAG} \cdot \text{CO}_2 \sim \text{P} + \text{NH}_3 \rightarrow \text{CPS} \cdot \text{NAG} \cdot \text{NH}_2\text{CO}_2\text{P}\]

(c) \[\text{CPS} \cdot \text{NAG} \cdot \text{NH}_2\text{C} \sim \text{OP} + \text{ATP} \rightarrow \text{NH}_3 \sim \text{C} \sim \text{OP} \text{(carbamyl phosphate)} + \text{ADP}\]

The reaction sequence can be summarized by the following:

\[\text{CO}_2 + \text{NH}_3 + 2 \text{ATP} \rightarrow \text{NH}_3 \sim \text{C} \sim \text{OP} + 2 \text{ADP}\]

The second step in the figure is the conversion of carbamyl phosphate and ornithine to citrulline, a reaction catalyzed by the mitochondrial enzyme ornithine transcarbamylase (OTC). The citrulline then formed moves out of the mitochondrion and into the cytoplasm, and indeed may leave the cell, to become a circulating substrate for ureogenesis in other tissues lacking enzymatic mechanisms to form citrulline. High levels of OTC relative to CPS have suggested that carbamyl phosphate synthetase and ornithine transcarbamylase are tightly linked (3).

The third step in the figure, is the synthesis of argininosuccinate from citrulline and aspartate. This step provides the critical link between the urea cycle and the citric acid cycle. Alanine, shown at the upper right as a prototype amino acid, is transaminated to pyruvate and gives up its amino group to ketoglutarate with the resultant formation of glutamate. The pyruvate is decarboxylated to enter the citric acid cycle as a two carbon fragment. The glutamate enters a transamination reaction with oxalacetate to form aspartate and to regenerate α-ketoglutarate. The aspartate so formed contains the second amino nitrogen destined for urea. In the presence of ATP,
aspartate combines with citrulline to form argininosuccinate, a reaction catalyzed by argininosuccinate synthetase.

To summarize step 3:

(a) Alanine $\rightarrow$ pyruvate + NH$_3$
(b) NH$_3$ + $\alpha$-ketoglutarate $\rightarrow$ glutamate
(c) Oxalacetate + glutamate $\rightarrow$ aspartate + $\alpha$-ketoglutarate
(d) Aspartate + citrulline + ATP $\rightarrow$ argininosuccinate + ADP

The fourth step, catalyzed by argininosuccinase, involves a cleavage of argininosuccinate to form fumarate and arginine. Fumarate returns to the citric acid cycle, and arginine is available for protein synthesis in extrahepatic tissues or further metabolism in the liver.

The fifth step, catalyzed by arginase, is the conversion of arginine to urea and ornithine, and the cycle is completed. That the components of the urea cycle may operate as unit was shown by an increase in all five enzymes of the urea cycle in livers of rats fed a high protein diet (7). In these animals, the activities of enzymes feeding into the urea cycle i.e., alanine amino transferase and aspartate amino transferase were enhanced concurrently. Such information indicating integration of the component steps is consistent with operation of the urea cycle as a metabolic pathway. The pace-setters for this pathway are generally considered to be the first step i.e., formation of carbamyl phosphate and the third step, the synthesis of argininosuccinate (8, 9). The rate-limiting step on the basis of tissue enzyme levels is argininosuccinate synthetase but what truly limits rates of ureogenesis is unknown, for perfusion studies have indicated that levels of argininosuccinate synthetase function at 60% capacity (10). Such observations suggest a possible limiting role of substrate availability, i.e., aspartate, citrulline, or ATP.

Unlike the liver, extrahepatic tissues contain only the last three enzymatic mechanisms for urea biosynthesis, and in brain, kidney and muscle, the biosynthesis of urea is dependent upon a source of citrulline, derived from the liver (11). Low levels of arginase in extrahepatic tissues suggest however, that portions of the urea cycle, i.e., argininosuccinate synthetase and arginino-

sucinate, may serve not as mechanisms for urea biosynthesis, but represent instead a means for utilizing imported citrulline to form arginine destined to participate in protein synthesis (12).

Until recently, significant interrelationships were thought to exist between the urea cycle enzymes and enzymes catalyzing the de novo biosynthesis of pyrimidines. It was shown that orotic acid destined to form uridine could be generated from aspartate and carbamyl phosphate via a cytoplasmic enzyme, aspartate transcarbamylase, at a stage during development when OTC levels were very low. The source of carbamyl phosphate remained, however unclear (13). As extra hepatic tissues were capable of synthesizing pyrimidines in situ (13), the possibility was entertained that exported carbamyl phosphate reached these tissues through the circulation (14). The tight linkage between CPS I and OTC in the mitochondria with predicted limited availability of carbamyl phosphate, and the absence of this substrate in the circulation prompted search for a carbamyl phosphate synthesizing enzyme in the cell cytoplasm. Subsequently, a cytoplasmic carbamyl phosphate synthetase was identified that did not require NAG as a cofactor and utilized glutamine instead of ammonia as a preferred nitrogen substrate (15, 16). The enzyme called carbamyl phosphate synthetase II has been demonstrated in virtually every growing mammalian tissue tested including the human lymphocyte (17, 18).

Like the enzymes of the urea cycle, the first enzymes of the pyrimidine synthesizing pathway move as a unit but follow rates of proliferative growth and are generally unaffected by diet. Developmental differences are also apparent. In the fetal rat, enzymes of the urea cycle develop during late intrauterine life, at a time when enzyme levels of the de novo biosynthesis of pyrimidines are steadily falling (19). Thus, despite the presence of similarly named enzymes (CPS I and CPS II) catalyzing the formation of an identical reaction product, carbamyl phosphate, the pyrimidine synthesizing and urea synthesizing pathways are quite different, and are linked only through a common requirement for ATP and aspartate.
The overall function of the urea cycle is to capture ammonia and represents an anabolic energy-requiring system permitting the formation of carbon skeletons required for oxidative metabolism without a concomitant burden of ammonia. It is apparent that the synthesis of one mole of urea requires a minimum expenditure of 3 moles of ATP. The metabolic fate of alanine by the following reaction sequences could provide the most economical disposition of available cellular energy.

(a) $2 \text{alanine} + 2 \alpha\text{-ketoglutarate} \rightarrow 2 \text{pyruvate} + 2 \text{glutamate} (\text{alanine amino transferase reaction})$.

(b) Glutamate $\rightarrow \text{NH}_3 + \alpha\text{-ketoglutarate} (\text{glutamate dehydrogenase reaction})$. The ammonia formed is available to enter step 1 of the urea cycle, and 2 moles of ATP are consumed.

(c) Glutamate $+ \text{oxalacetate} \rightarrow \text{aspartate} + \alpha\text{-ketoglutarate} (\text{aspartate amino transferase})$. The aspartate formed is now available to enter step 3 of the urea cycle, and 1 mole of ATP is consumed.

(d) The 2 moles of pyruvate generate 30 moles of ATP, and net energy available from catabolism of 2 moles of alanine is 30–3 or 27 moles of ATP.

As energy is required to dispose of excess ammonia, it is apparent that a source of ATP must be available for the urea cycle to work. Thus, during embryogenesis, a functioning citric acid cycle must precede the development of the urea cycle. Before development of the urea cycle, fetal tissues have two potential mechanisms for ammonia capture. The first is the conversion of $\alpha\text{-ketoglutarate}$ and ammonia to form glutamate (via glutamate dehydrogenase), and the second is the conversion of glutamate to glutamine (via glutamine synthetase). As neither reaction generates substrate for the citric acid cycle, fetal oxidation is dependent on carbon fuels derived from the mother i.e., glucose, lactate, and during maternal fasting, ketone bodies. Lactate has recently been shown to be an important fuel in the fetal sheep (20) and rat (21), and the placenta has emerged as a potentially important source (22). The acquisition of a functioning urea cycle permits the fetus to exploit amino acids as additional fuel sources to meet increased oxidative demands during rapid growth. In the human, fetal liver is capable of synthesizing urea as early as the 16th week (23), and all five enzymes have been shown to be present by the 50th day of pregnancy (24). Thus, accelerated increases in body mass between the 50th and 110th day of gestation are accompanied by enhanced levels of urea in fetal liver. The adaptive capabilities of the urea cycle enzymes are acquired at this time, for with the slower fetal growth rates during the second trimester, and presumably diminished rates of amino acid turnover, concentrations of urea synthesizing enzymes decline and urea levels in the liver fall concordantly.

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References

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