

Chapter 9

## The capacity of urea synthesis in rats: regulation and physiological importance

HENDRIK VILSTRUP, BENT ADEL HANSEN, THOMAS ALMDAL, KITT FALK PETERSEN, INGA SIGSGAARD AND HENRIK ENGHUSEN POULSEN

*Division of Hepatology, Rigshospitalet A-2151, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark*

### Summary

A method is described for determination of the Capacity of Urea-N Synthesis (CUNS) in rats that allows studies of the regulation of urea synthesis independent of changes in amino acid concentrations. Experiments are reported showing that CUNS is increased by glucagon and cortisol, and decreased by insulin and carbohydrates. CUNS depends on the liver mass and is restored more quickly than other liver functions during liver regeneration. CUNS increases with high protein feeding and decreases with protein depletion. CUNS is increased in experimental diabetes with protein wasting. The hypothesis is presented that changes in regulation of urea synthesis are of primary importance for tissue protein synthesis.

### Zusammenfassung

**Die Kapazität der Harnstoff-N-Synthese bei Ratten: Regulation und physiologische Bedeutung.** In der vorliegenden Arbeit wird ein Verfahren zur Bestimmung der Harnstoff-N-Synthesekapazität (HNSK) bei Ratten beschrieben; das Verfahren ermöglicht es, die aminosäurenunabhängige Regulation der Harnstoffbildung zu untersuchen. Es wird über Experimente berichtet, aus denen hervorgeht, daß Glukagon und Kortisol die HNSK steigern, während Insulin und Kohlenhydrate zu einer Einschränkung führen. Die HNSK richtet sich nach der Lebermasse und normalisiert sich bei Leberregeneration schneller als andere hepatische Funktionen. Sie nimmt im Gefolge einer proteinreichen Ernährung zu und bei Proteinentzug ab. Ein experimenteller Diabetes mit Eiweißverlust vergrößert die HNSK. Wir unterstellen, daß Regulationseinschränkungen der Harnstoffbildung sich auf die Proteinsynthese der Gewebe in hohem Maße auswirken.

## I. Introduction

Since most amino-N not utilized for protein synthesis is eventually eliminated by incorporation into urea, studies on the rate of urea synthesis provide a means of gaining insight into the 'terminal' part of the dynamics underlying the N-homeostasis of the organism. In vitro studies have shown that the activity of the urea cycle enzymes is susceptible to a variety of physiological changes. However, because of the marked dependence of urea synthesis on amino acid supply it has proven difficult to examine the regulation of the kinetics of urea synthesis *in vivo*.

This survey describes a method for measuring the urea synthesis rate in rats, the Capacity of Urea-N Synthesis (CUNS), under conditions where substrate drive is standardized. The effects of various hormones, carbohydrates and pathological conditions on CUNS are reported. Finally, the hypothesis is presented that urea synthesis plays an active role in whole-body N homeostasis.

## II. Method

The urea synthesis rate was measured in rats weighing about 200 g as the accumulation rate in the urea space (i.e. the total body water) corrected for loss of urea by gut bacterial hydrolysis. Measurement of the urinary excretion requires bladder catheterization and quantitative collection of small urine volumes, which was found to be very uncertain. Urinary excretion was therefore avoided by retroperitoneal nephrectomy immediately before the synthesis rate was measured. The nephrectomy in itself did not influence the urea synthesis rate. The urea space (measured by i.v. urea bolus injection) and the gut hydrolysis (measured by a [<sup>14</sup>C]urea tracer method) were not influenced by the different conditions under which urea synthesis was examined.

The rate of urea synthesis depends on the concentration of amino-N in the portal blood. Thus, it can be expected that determination of the rate reflects the supply of amino-N rather than the physiological regulation of urea synthesis. To overcome this problem it is necessary to know the relationship between the urea synthesis rate and blood amino-N concentration, i.e. the kinetics of urea synthesis. For this purpose alanine was chosen as amino-N source. Alanine was administered via a central vein to avoid uncertainty due to intestinal absorption and to ensure that the concentration in the peripheral blood, from which samples were taken, was not markedly different from the portal blood concentration.

Determination of the urea synthesis rate at a given  $\alpha$ -amino-N concentration requires that the concentration is constant in the time period during which urea accumulation is measured (50 min). This was ensured by a priming dose of alanine, determining the amino-N concentration, followed by a constant infusion maintaining this concentration. The rate of the constant infusion was adjusted before the measurement of the urea synthesis rate was started to ensure a steady state. The adjustments were made according to empirical criteria and were based on rapid analysis of blood  $\alpha$ -amino-N concentration (7 min) by a modification of the dinitrofluorobenzene method (increased reaction temperature). Thus one rate of

urea synthesis at one  $\alpha$ -amino-N concentration could be determined in each rat. For details, see Ref. 1.

The kinetics of urea synthesis, as based on 24 experiments at different  $\alpha$ -amino-N concentrations, is shown in Fig. 1: at low concentrations there is a very steep increase in synthesis rate with increasing concentrations; at high concentrations there is a gradual decrease in synthesis rate with increasing concentrations, and in an intermediate interval the synthesis rate is near maximum and does not change appreciably with changes in amino acid concentration. Mathematically, this type of kinetics can be described as barrier-limited substrate inhibition kinetics. Further analysis shows that the rate of urea synthesis deviates less than 5% from the theoretical zenith of the curve within the concentration interval 7.3–11.6 mmol/l. The rate of urea synthesis measured at  $\alpha$ -amino-N concentrations within this interval represents the Capacity of Urea-N Synthesis (CUNS) and is independent of substrate drive. CUNS can thus be used for investigation of physiological regulation of urea synthesis.

### III. Regulation of CUNS by hormones and carbohydrates

In vitro investigations of isolated rat liver enzymes have shown that the activity of the urea cycle enzymes can be increased by high concentrations of glucagon and cortisol. The urinary output of urea has been shown to decrease when insulin is given. Glucose is reported to have the same effect, possibly via release of insulin. Xylitol works as glucose, but does not in itself stimulate insulin release.

The possible regulatory effects of these factors on urea synthesis in vivo were examined in a series of experiments which attempted to study the effect of each of them independently.

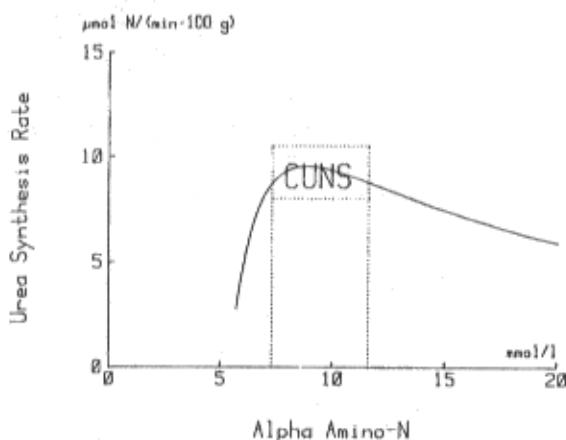


FIG. 1 Relationship between blood  $\alpha$ -amino-N concentration and urea-N synthesis rate in rats. Dotted lines indicate the concentration interval 7.3–11.6 mM in which the rate of urea synthesis was within 5% of its theoretical maximum.

Exogenous hyperglucagonaemia was created by daily s.c. injection of 20  $\mu\text{g}$  of zinc glucagon. CUNS was measured after 2, 8, and 14 d in groups of 6–10 rats [2]. There was an interval of 3 h between glucagon dosage and the determination of CUNS, at which time both glucagon, insulin and glucose concentrations were normal. CUNS increased with time and tripled after 14 d. Infusion of crystalline glucagon to control rats did not change CUNS. Thus glucagon stimulates CUNS in a time-dependent manner. This does not presuppose simultaneous hyperglucagonaemia, which suggests that the effect depends on enzyme induction.

The effect of glucocorticosteroids was studied by daily i.p. injection of 0.2 mg of dexamethasone to groups of 5–8 rats. Glucagon fell slightly, insulin increased 7–8-fold, and blood glucose increased slightly. After 2 d CUNS doubled, and normalized after 14 d. Since this effect is not explainable by changes in glucoregulation (cf. below), cortisol in itself transiently up-regulates CUNS [3]. During the dexamethasone treatment the animals lost body weight, most markedly during the early phase. This finding is discussed later.

The study on insulin effects required maintenance of euglycaemia by feed-back-controlled infusion of glucose, i.e. a euglycaemic 'clamp' technique. The endogenous hormonal responses were controlled by infusion of somatostatin, which in itself did not influence CUNS. Insulin was infused to different degrees of hyperinsulinaemia in 26 rats. CUNS decreased linearly with insulin concentrations up to 200 mU/l (normal fasting value 15). At higher insulin concentrations CUNS did not decrease further [4]. The decrease of CUNS by insulin is dose-dependent and independent of glucose. The effect is instantaneous and therefore does not depend on changes in the amount of urea enzymes.

During the glucose study the hormonal responses were controlled by somatostatin. Different blood concentrations of glucose were obtained by the hyperglycaemic 'clamp' technique in 20 rats (unpublished data by B.A. Hansen and H. Vilstrup). Hyperglycaemia up to 8 mmol/l decreased CUNS linearly to 60%,

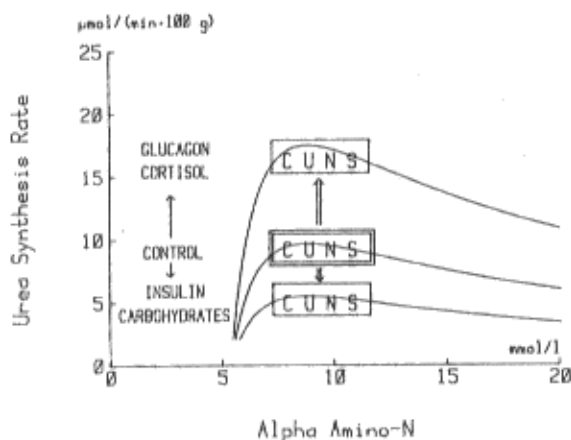


FIG. 2 Effects of hormones and carbohydrates on CUNS. The changes in CUNS imply that the urea synthesis rate at any amino acid concentration is changed.

whereas higher glucose concentrations did not decrease CUNS further. The effect is independent of insulin and glucagon and is immediately exerted.

Xylitol increased glucose and thereby insulin. Insulin was controlled in groups of 6 rats by somatostatin and the glucose effect was compensated for by inclusion of control groups in which glucose was clamped to the same concentration as that resulting from xylitol. The xylitol blood concentration was clamped at 1 mmol/l (the recommended therapeutic concentration) and 5.5 mmol/l. Xylitol decreased CUNS in a dose-dependent manner to 30% at the highest concentration (unpublished data by B.A. Hansen, T. Almdal and H. Vilstrup). The glucose effect can account for only half of this decrease.

The results are summarized in Fig. 2. Several hormones and carbohydrates regulate urea synthesis independently, in different directions, with different time-dependence, and with different dose-dependence. Combinations of these and other as yet unidentified effects are responsible for the pattern of changes in urea synthesis under different physiological conditions.

#### IV. Changes in CUNS under different conditions

The relationship of CUNS to liver mass, function, and regeneration was studied by partial hepatectomy of groups of 6–8 rats. Immediately following removal of 30–90% of the liver CUNS was proportional to the weight of the remaining liver [5]. This shows that CUNS depends on the liver mass and suggests a role for CUNS as a measure of liver function, provided that the factors mentioned previously have predictable effects. During the early phase (6 h) of liver regeneration after 70% hepatectomy CUNS increased to two-thirds of control values, while the liver weight was still one-third of normal. This contrasts with the course of other liver functions, the galactose elimination capacity and the clearance of antipyrine, both of which were restored more slowly than the liver weight and remained subnormal after 10 d [6]. During regeneration the liver follows a metabolic strategy that gives priority to amino-N conversion. This may be due to other factors besides those described in the previous section.

CUNS increased by one-third after a 24 h fast, but normalized during more sustained food deprivation (4 d) [7]. This is at variance with the galactose elimination capacity and the clearance of antipyrine, which both show later decreases.

The influence of a catabolic state was further studied by induction of diabetes mellitus in groups of 8 rats by i.v. injection of streptozotocin. Total body N content fell to less than 60% in the course of 4 weeks although the diabetic rats ate the same as the control animals [8]. CUNS increased time-dependently to a maximum of 2.5-times control [9]. This may be due to the marked and sustained diabetic hyperglucagonaemia. The relationship to protein wasting is discussed in the next section.

Increased dietary protein intake has been shown to increase urea cycle enzyme activity *in vitro*. The importance for CUNS of varying the protein intake was examined in groups of 6 rats. They were given 10, 17 (normal) or 57% of the calories as protein and studied after 14 d. CUNS was reduced to 70% by the low-protein diet

and doubled by the high-protein diet (unpublished data by K.F. Petersen). Since CUNS is a substrate-independent parameter these changes do not merely reflect different amounts of N that had to be disposed of. The adaptive changes in urea synthesis *in vivo* are probably due to regulation of enzyme activity, possibly by one or several of the factors discussed previously.

These results show that the dynamics of urea synthesis change markedly over a wide spectrum of pathological conditions. Some of the changes are explainable by known regulators. The relationship to tissue amino-N release and uptake is not simple, in that the changes in CUNS are not secondary to different amino-N loads on urea synthesis.

### V. A primary role of urea synthesis for N homeostasis?

A change in CUNS implies that the amount of urea synthesized at a given substrate drive is changed: if CUNS doubles, about twice as much urea is synthesized at any amino acid concentration (Fig. 2). The regulatory factors and the conditions described above are responsible for the fact that urea synthesis is changed in a way which is independent of changes in blood amino acid concentration. Consequently, changes in CUNS influence the blood free amino acid concentration rather than vice versa.

The turnover of the blood free pool of amino acids is very rapid — for certain essential amino acids and without exogenous supply a matter of minutes. Evidently, the concentration of amino acids in the blood is influenced by the balance between

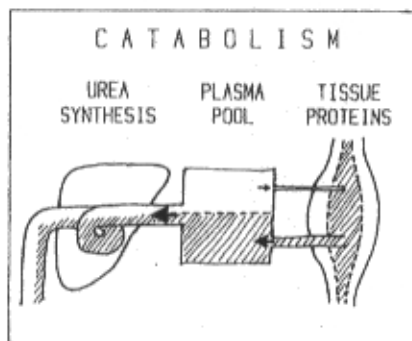


FIG. 3 Hypothetical flux of amino-N in a catabolic state. The hatched area represents amount of amino-N. The diameters of the tubes indicate supposed flux rates in the catabolic state. Draining of amino acids from the plasma pool by the liver has the largest bore, and the contribution of amino acids to the pool by the tissue proteins has a larger bore than the removal of amino acids from the pool by the tissues. The result is that the pool is half-emptied by the liver to a degree where the flow into tissue proteins nearly ceases. The large flow of amino acids from the tissue proteins does not increase the plasma pool of amino acids, since the liver drains the pool even faster. The effect of this pathological combination of unusual fluxes is that tissue amino-N disappears from the body, as shown by the diminished tissue protein content.

protein synthesis and break-down in the tissues. Conversely, however, the blood free amino acid concentrations may well influence this balance: depletion of the blood pool of amino acids will impede protein build-up – or may possibly increase protein breakdown.

It is thus conceivable that an increase in CUNS will impair tissue nitrogen balance because the increase in urea synthesis rate at any given blood amino acid concentration leads to elimination of amino-N which is then lost for protein synthesis.

Eventually, however, the rate of urea synthesis is still determined by the amino acid concentration, even if CUNS is increased. Therefore the synthesis rate will decrease when the amino acid concentration falls, and a new N homeostasis is attained at another metabolic set point, where protein build-up is less favoured than protein breakdown. It is as yet unproven that this condition by itself can lead to significant protein wasting.

If, however, CUNS increases simultaneously with a pathological increase in protein breakdown, a vicious circle is established in which protein wasting becomes life-threatening: amino-N is virtually blown out of the tissue and consumed by the liver even faster.

Examples of this are the body mass wasting in experimental diabetes and during corticosteroid treatment.

The hypothesis is illustrated in Fig. 3. We hypothesize that changes in the kinetics of urea synthesis may be of primary importance for protein wasting in chronic and acute disease. Changes in urea synthesis may be secondary to changes in the interplay between several hormones and substrates.

## References

- 1 Hansen, B.A. and Vilstrup, H. (1985) *Scand. J. Clin. Lab. Invest.* 45, 315–320.
- 2 Petersen, K.F., Hansen, B.A. and Vilstrup, H. (1987) *Horm. Metab. Res.* 19, 53–56.
- 3 Sigsgaard, I., Almdal, T., Hansen, B.A. and Vilstrup, H. (1987) *J. Hepatol.* 4 Suppl. 1, S43.
- 4 Hansen, B.A., Krog, B. and Vilstrup, H. (1986) *Scand. J. Clin. Lab. Invest.* 46, 599–603.
- 5 Hansen, B.A. and Poulsen, H.E. (1986) *J. Hepatol.* 2, 468–474.
- 6 Hansen, B.A. and Poulsen, H.E. (1986) *Scand. J. Clin. Lab. Invest.* 46, 233–237.
- 7 Hansen, B.A. and Poulsen, H.E. (1986) *Clin. Nutr.* 5, 213–216.
- 8 Almdal, T. and Vilstrup, H. (1987) *J. Hepatol.* 4 Suppl. 1, S1.
- 9 Almdal, T.P., Petersen, K.F., Hansen, B.A. and Vilstrup, H. (1986) *Diabetologia* 29, 812–816.