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Francesco Cedrangolo, Gennaro Illiano,
Riccardo Cortese

Further studies on the role of free ammonia in urea biosynthesis

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Biochimica. — *Further studies on the role of free ammonia in urea biosynthesis* (*). Nota di FRANCESCO CEDRANGOLO, GENNARO ILLIANO e RICCARDO CORTESE, presentata (**) dal Socio F. CEDRANGOLO.

RIASSUNTO. — Allo scopo di chiarire se l'ammoniaca libera funge da intermediario nella biosintesi dell'urea, è stata dosata l'NH_3 prodotta *in vitro* in diversi organi e tessuti di ratto tenuti a dieta normale ed iperproteica. I risultati ottenuti, paragonati con i dati di escrezione di urea nei due gruppi di animali, suggeriscono la possibilità di un ciclo dell'urea in cui l'NH_3 non funge da precursore. Infatti, mentre la produzione di ammoniaca è la stessa nei due gruppi di animali, la quantità di urea escreta dagli animali a dieta iperproteica supera largamente quella escreta dagli animali a dieta normale.

According to studies of Cedrangolo *et al.* [1–6] free ammonia is not an intermediate in urea biosynthesis in mammals. On the other hand the urea cycle, as it is generally accepted, implies the derivation of at least one nitrogen atom of urea from free ammonia; the origin of the second nitrogen being not clear. In fact, this last could originate: (*i*) from the amino nitrogen of amino acids through several transamination reactions without the participation of free ammonia; (*ii*) from free ammonia through the reductive amination of oxoglutarate: the glutamate formed could originate aspartate *via* transamination.

To further investigate this problem, a series of experiments have been devised: in several tissues of rats fed with normal or high protein diets the ammonia formed *in vitro* has been measured. The results have been compared with the data of urea excretion *in vivo* in animals under identical conditions.

MATERIALS AND METHODS

All the chemicals employed were obtained from commercial sources and were analytical grade. Ammonia-free distilled water was obtained by processing glass-distilled water through acid-treated permutite.

Nessler's reagent was prepared according to Vanselow [7]. Saturated borate–NaOH solution (pH 10.8) was prepared according to Reinhold and Chung [8]. Ammonia produced was analysed by Conway's microdiffusion technique modified by Cedrangolo *et al.* [9]. A special rotator was used to hold microdiffusion bottles (50 ml), this device gives high performance through well controlled temperature levels and rotation speed (50 *rpm*). The pH, during microdiffusion, was controlled at 11 ± 0.2 to obtain a complete reco-

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very of free ammonia present in the tissue and to avoid formation of artifactual ammonia from amides, proteins, etc. [*l. c.* 9].

Wistar male rats were obtained from a breeding farm (Morini, Reggio Emilia, Italy). The animals were divided in two groups: one fed with normal diet, the second with high protein diet (boiled horse meat).

Freshly excised liver, brain, and kidney were gently blotted on filter paper, then rinsed with cold buffer and immediately homogenized (15 %, *v/v*) in phosphate buffer (KH_2PO_4—K_2HPO_4) 0.1 M at pH 7.4. The whole procedure was performed in the cold room at 2° C. The homogenate was made in a glass Potter-Elvehjem apparatus with teflon pestle at 1,000 *rpm* for 2 min. 1 ml samples were transferred into small flasks and placed in a Dubnoff shaking-incubator at 37° C. After 10 minutes of incubation the ammonia was measured by microdiffusion and the values were corrected for the ammonia present at zero time.

Results and Discussion

In Table I are reported the results obtained from the three organs. The results are expressed as NH_3 formed/kg body weight in the course of 60 minutes: for the calculations the average weights of the organs per Kg of animal have been taken into account [10]. An increase of ammonia production is detectable essentially in the liver and, then, also in the brain of the animals fed with an high protein diet, while the ammonia formation decreases in the kidney of the same group of rats.

Table I

Ammonia production from several tissues of rat.

Organ	NH_3 produced/hr/kg body weight (μmoles)	
	Rats fed with normal diet	Rats fed with high protein diet
Liver	1,093 ± 61 (*)	1,338 ± 68
Brain	383 ± 18	468 ± 20
Kidney	635 ± 21	391 ± 18

(*) Standard deviation.

If we compare the sum of the data reported in Table I with the urea excretion studied *in vivo* [11] in animals in the same feeding conditions, we can observe (Table II) that the difference in ammonia formation (86 μmoles) does not account for the large increase of urea excretion (3,631 μmoles

as NH_3). Even if we consider only the liver, in which ammonia production is particularly increased in the group fed with high protein diet, the difference (245 μmoles) is still much lower compared with the amount of extra-urea.

TABLE II

Comparison between urea excretion and ammonia formation in rat.

	Rats fed with normal diet	Rats fed with high protein diet
NH_3 formed from 3 organs/hr/kg body weight (μmoles)	2,111	2,197
Urea excreted expressed as NH_3/hr/kg body weight (μmoles)	2,604	6,235

The reported results seem to be indicative for a mechanism of urea formation without the participation of free ammonia. It should be noted, moreover, that ammonia has been determined only in three organs, where it would be more correct an evaluation of the ammonia formed in the whole rat. On the other hand, liver, kidney and brain seem to be the organs chiefly involved in urea formation.

Even if a more general conclusion cannot be drawn only from the present experiments, it should however be emphasised that the results are in close agreement with previous data obtained in this Laboratory [*l. c.* 1–6, 11].

Experiments with ^{15}N–amino acids are in progress in this Laboratory to further clarify the problem.

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