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Presence of a partial urea cycle in the leech, *Poecilobdella granulosa*

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Abstract. Ornithine carbamoyltransferase (OCT) and arginase, but not arginine synthetase (AS), were detected in the body wall and gut tissues of the leech. The activities of these enzymes were not altered by starvation. The high activity of arginase in body wall is probably due to the association of the latter with botryoidal tissue. Hirudineans, which evolved from oligochaete ancestors, appear to have lost the citrulline-arginine segment of the urea cycle due to their ammonotelic mode of nitrogen excretion.
Key words. Urea cycle; leech; botryoidal tissue; hirudineans; evolution.

Annelids are primarily ammonotelic [4, 5]. However, earthworms are known to switch over to ureotelism during starvation and considerable quantities of urea are present in the excreta of leeches and coelomic fluid of polychaetes [5]. Since uricolytic enzymes are absent in all members of Annelida [4], purine degradation does not seem to contribute to the excretory urea in these animals. The observation of Robin et al. [6] that in leeches labelled amidine from arginine appears in urea prompted Needham [5] to postulate the possible presence of urea cycle enzymes in hirudineans. However, the earthworm, *Lumbricus terrestris*, is the only annelid where a functional urea cycle has been demonstrated to date [7, 8]. Representatives of Polychaeta and Hirudinea have not been hitherto investigated for urea cycle enzymes. We report here that only a partial urea cycle is present in the Indian cattle leech, *Poecilobdella granulosa*, and that the activities of these enzymes, unlike those of the earthworm, are not altered in response to starvation stress.

A single blood meal sustains the cattle leech, *P. granulosa*, for as long as six months before the effects of starvation are noticeable [9]. Leeches were collected after a blood meal and used in experiments within a couple of weeks (fed group) or eight months later (starved group). Animals with empty and blood-filled alimentary canals were discarded from the fed and starved groups respectively. Body wall and gut tissues were homogenized at 0 °C in 9 volumes of 0.1 % acetyltrimethylammonium bromide. Acid-washed sand (BDH, England) was used in the homogenization of body wall. The homogenates were centrifuged at 4 °C, 15,000 × g for arginase and 4000 × g for ornithine carbamoyltransferase (OCT) and arginine synthetase (AS; combined activity of argininosuccinate synthetase and argininosuccinate lyase). The enzyme activities in the supernatants were assayed, after appropriate dilution, as described earlier [10, 11].

Arginase, which catalyzes the conversion of arginine to ornithine and urea, and OCT, which catalyzes the conversion of ornithine and carbomylphosphate to citrulline, were present in both body wall and gut tissue of the leech (table). The AS system, which forms arginine from citrulline, aspartate and ATP, could not be detected in either tissue. More concentrated homogenates and longer incubation periods were employed to confirm the apparent absence of this enzyme system in leech tissues. Our inability to detect AS in leech tissues is not due to the inadequacy of the assay method employed, because the method yielded enzyme activity levels in frog and rat livers similar to those reported in the literature [12].

OCT and arginase activities in leech tissues were lower than those in the earthworm gut [7]. In the leech, the activity levels of arginase were 11-fold higher in the body wall than the gut, while the levels of OCT were similar in both tissues (table). The two tissues in the polychaete, *Arenicola marina*, exhibit about the same level of arginase activity [13]. In the earthworm, on the other hand, both enzyme activities are several times higher in the gut than in the body wall [7, 14]. Most of the arginase activity in the earthworms is concentrated in the chloragog tissue, which forms a compact layer surrounding the gut, while OCT is

Urea cycle enzyme activities (μmoles product/h/g tissue) in leech tissues

Enzyme	Body wall		Gut	
	Fed	Starved	Fed	Starved
Carbamoylphosphate synthetase	NA	NA	NA	NA
Ornithine carbamoyltransferase (OCT)	82 ± 6 (72–90)	91 ± 1 (90–92)	84 ± 3 (80–89)	95 ± 3 (90–99)
Arginine synthetase (AS)	BLD	BLD	BLD	BLD
Arginase	225 ± 87 (94–319)	210 ± 70 (113–285)	20 ± 8 (12–30)	18 ± 7 (12–30)

NA, not assayed; BLD, below the level of detection; the values given are the means ± SD of assays carried out on tissues from 6 leeches with ranges in parentheses below.

distributed both in the chloragog tissue and intestinal epithelium [8]. The botryoidal tissue of leeches, which corresponds to the chloragog tissue of oligochaetes in origin and function, occupies the dorsal regions of the coelome immediately below the body musculature [15, 16]. This association of the botryoidal tissue with the body wall musculature might explain the distribution pattern of the two enzymes in gut and body wall of the leech.

Unlike the earthworm, where the activities of all the urea cycle enzymes increase in response to fasting [7, 8], OCT and arginase activities in both tissues of the leech remained unaltered eight months after the last blood meal (table). In addition, the AS system was not detectable in the tissues of the starved leeches. This difference between earthworm and leech is consistent with the dependence of oligochaetes on body protein and of hirudineans on stored carbohydrate during starvation [5].

Of the two enzymes of the urea cycle present in the leech, arginase might serve to generate ornithine as a precursor for the synthesis of polyamines and hirudonine [17]. The metabolic significance of citrulline, the product of OCT reaction, is not clear in these animals. Since the urea cycle serves the dual function of ammonia detoxification and arginine biosynthesis [12], the incomplete urea cycle makes arginine an essential amino acid for leeches. The urea present in the excreta of these animals is probably formed by the action of arginase on exogenous arginine. An apparently incomplete urea cycle, in which the citrulline-arginine segment of the cycle is missing, has earlier been reported in other ammonoteles like crayfish and channel catfish [18, 19].

The urea cycle of animals has evolved from the arginine biosynthetic pathway of prokaryotes [20]. The sequence homologies shared between the mammalian urea cycle enzymes and microbial arginine biosynthetic enzymes point out their evolutionary relatedness [21]. The widely-held view in animal phylogeny is that the annelid-arthropod, molluscan and echinoderm-protochordate-vertebrate lines have branched out from the planuloid-flatworm stem [22]. The presence of a functional urea cycle in planarians and representatives of each of these phylogenetic lines, viz., earthworm, gastropod snails and vertebrates suggests the evolutionary continuity of this biochemical pathway from its basic function of arginine biosynthesis in microorganisms to its specialized role in ammonia detoxification in ureotelic animals [12]. The urea cycle is either completely or partially absent in ammonoteles, uricoteles and guanoteles [10–12, 18, 19] and this does not seem to be due to gene deletion [23]. While the genetic potential for the cycle has presumably persisted throughout the animal kingdom, it appears to be expressed only in ureotelic and ureosmotic species. The results of studies on the urea cycle in annelids, though restricted to the earthworm and leech, are consistent with these ideas. Although it is debated whether polychaetes gave rise to oligochaetes or vice-versa [24], it is generally agreed that hirudineans have evolved from an oligochaete stock [16, 22, 24]. Since the oligochaete ancestors of hirudineans probably possessed a complete urea cycle, the apparent absence of a functional urea cycle in leeches might be the result of ammonotelism. The loss of the urea cycle in toto or in part, depending on the metabolic requirement of the intermediates, appears to have occurred many times in animal evolution as a consequence of non-ureotelic modes of nitrogen excretion.

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Do taste receptors respond to perturbation of water structure?

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Abstract. The pmr spin-spin pulse relaxation times (T_2 values) of the L-amino acids are examined in relation to their taste threshold values. There is an inverse trend between T_2 value and threshold value with a good correlation for amino acids whose natural pH is close to neutrality. These results may indicate that taste receptors respond to perturbation of water structure.

Recent interest in the solution properties of small carbohydrate molecules in relation to their sweet taste[1,2] and indeed, in the 'packing characteristics' of all sapid molecules in water as predictors[3] of their taste quality, has refocussed attention on water structure[4–7]. A heavily hydrated solute, such as a sugar or an amino acid is likely to interfere, substantially, with hydrogen-bonding between water molecules, and this process may be of particular significance in the localised micro-environment of receptors, where water activity is minimal[1,3]. The response of a receptor to water structure perturbation, rather than to stimulus molecular structure might be manifested more in terms of detection threshold (see ref. 17 below), than recognition threshold or supra-threshold magnitude estimation, because the low concentration of stimulus involved avoids the complexity of recognition and memory processes.

Materials and methods

T_2 values were determined using a Bruker Minispec NMR Spectrometer, a low resolution instrument (20 MHz) operating at 40 °C. The Carr-Purcell, Meiboom Gill (CPMG) sequence was used to measure T_2 values. The T_2 values thus obtained reflect the exchange of spin energy between protons which in turn depends on their structural environment within the molecules. Amino acids were reagent grade chemicals obtained from Sigma Chemical Company, Poole, Dorset, except for L-alanine, L-glutamic acid L-histidine and L-leucine which were obtained from BDH, Poole, Dorset. Water used was 'Water for HPLC' (BDH).

Results and discussion

The table ranks fourteen L-amino acids in descending order of pmr spin-spin relaxation times (T_2 values) and again in ascending order of detection thresholds. The two rankings are not identical but similar. The T_2 ranking reflects differences in overall energy exchange between water protons, and in this respect is an indicator of the solute's influence on water structure.

The table lists the natural pHs for the amino acid solutions. This demonstrates that amino acids with a high or low pH have the lowest detection thresholds. T_2, which is dependent upon pH (figs 1 and 2), differentiates these amino acids. High or low pH would of course distinguish

Ranking of the L-amino acids according to spin-spin relaxation times, T_2 at a 2-ms 180° pulse separation and detection threshold values Cd.

Cd[17] (mM/l)	pH	T_2 (sec)
Glu 0.063	3.4	Arg 3.9
Lys 0.708	9.6	Lys 3.8
Arg 1.20	10.1	Glu 3.3
His 1.23	7.3	His 3.2
Trp 2.29	6.0	Trp 3.2
Met 3.72	5.8	Pro 3.1
Val 4.16	6.1	Met 3.0
Leu 6.45	5.9	Phe 3.0
Phe 6.61	6.3	Val 2.9
Ile 7.14	6.4	Leu 2.9
Gln 9.77	5.7	Gln 2.9
Pro 15.1	6.4	Ile 2.9
Ala 16.2	5.9	Ala 2.9
Ser 20.9	6.3	Ser 2.8

The T_2 values were measured on a proton magnetic resonance spectrometer operating at a frequency of 20 MHz and at a sample temperature of 40 °C. The CPMG sequence was used to measure T_2[18].