UREA RETENTION IN THE HEMOLYMPH OF THE SHORE CRAB *CARCINUS MAENAS* DURING PROLONGED STARVATION AND A FIRST APPROACH TO IDENTIFYING A BRANCHIAL UREA TRANSPORTER

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It is well known that water-breathing animals excrete their nitrogenous end products predominantly in the form of ammonia. In the intertidal shore crab *Carcinus maenas,* 86% of the total N-excretion is in the form of ammonia whereas the excretion of urea and uric acid is very small (3% and 0.7% respectively) (Needham, A.E. Phys. Comp. Oec. IV: 209-239, 1957). In the present study, urea concentrations in the hemolymph of starved *C. maenas* were investigated. Crabs were acclimated in recirculating diluted seawater (10 ppt) over a period of at least 3 weeks and hemolymph samples were taken at different time periods after a final feeding. Samples were then analyzed for their ammonia and urea content (Boehringer Mannheim).

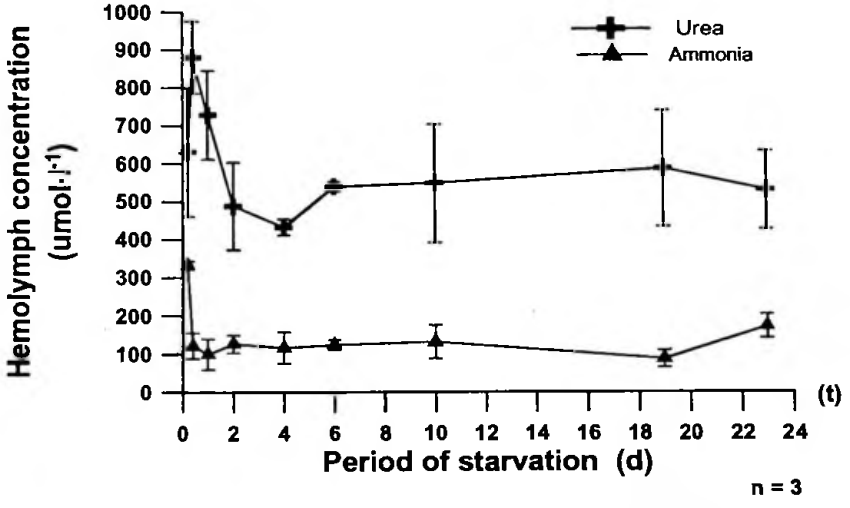


Fig. 1: Concentration of ammonia and urea in the hemolymph of the shore crab *Carcinus maenas* after different time periods of starvation.

Four hours after feeding, an initial peak of hemolymph ammonia concentration occurs followed by an increase of hemolymph urea (Fig. 1). The less toxic urea possibly functions as a buffer to keep the internal level of toxic ammonia low, a function already suggested by Spargaaren (Nether. J. Sea. Res. 15: 273-283, 1982) when crabs were exposed to high environmental ammonia concentrations. However, after 24 h to 48 h of starvation, ammonia and urea concentrations in the hemolymph remained constant during the following 3 weeks. Urea apparently does not serve as a nitrogen source during this period of nitrogen shortage.

It is remarkable that relatively high urea concentrations were maintained in the hemolymph. Because excretion of urea is low in the shore crab, epithelia facing the environment, in particular the moderately leaky gills and the extremely leaky antennal gland

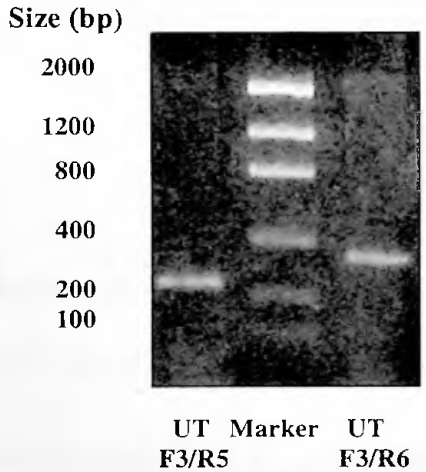
system, most likely bear a mechanism to retain urea. A putative candidate serving this task is the urea transporter (UT) already described in vertebrates (Smith, C.P. and Wright, P.A., Am. J. Physiol. 276: R622-R626, 1999). To investigate this possibility, total RNA was isolated from spiny dogfish (*Squalus acanthias*) and from gills and antennal gland of brackish water acclimated shore crabs *C. maenas*. Poly-A mRNA was reverse transcribed to single-stranded cDNA using an oligo-dT primer. Design of the degenerate primers (UTF3, UTR5 and UTR6;

Sense primer		***Anti-sense primers***	
UT F3	5'-tayggitgygayaayccitgg-3'	**UT R5**	5'-gciaritrrtgigtytgcca-3'
		UT R6	5'-caraaiggccaigtrca-3'

Fig. 2: Degenerate primers used in PCR experiments. h=a/c/t, i=a/c/g/t, r=a/g, y=c/t

see Fig. 2) were based on conserved regions aligned using published urea transporter (UT) sequences from several vertebrates (incl. spiny dogfish *S. acanthias*). Putative UT fragments were amplified by the polymerase chain reaction (PCR) employing total cDNA as a template. The PCR products from dogfish kidney exhibited predicted sizes of the known urea transporter published by Smith and Wright (1999) (Fig. 3). The PCR product UTF3/R5 was purified and sequenced at MDIBL's Marine DNA Sequencing Center. GenBank analysis revealed a 76 amino acid fragment showing a high homology to other published urea transporters.

Fig. 3: RT-PCR products separated on a 1.5% ethidium bromide gel. For RT-PCR degenerate primers UTF3/R5 and UTF3/R6 and total cDNA from shark kidney (annealing temperature = 45 °C) were employed.



These working degenerate primers were employed in an attempt to amplify related cDNA sequences from *C. maenas* tissue (gill and antennal gland), testing low stringency annealing temperatures (38-50°C) but without any success (data not shown). Retention of urea by the shore crab may occur by a mechanism other than the urea transporter cloned in vertebrates, or the crustacean UT may differ substantially in sequence.

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