THE PHYSIOLOGY OF THE ANTENNAL GLAND OF
CARCINUS MAENAS (L.)

V. SOME NITROGENOUS CONSTITUENTS IN THE BLOOD AND URINE

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INTRODUCTION

It is now well established that the antennal glands of Crustacea, both marine and fresh water, are concerned with regulation of the ionic composition of body fluids (Robertson, 1957; Shaw, 1960; Lockwood, 1962; Potts & Parry, 1964). This activity is considered by some to be the main function of the antennal gland (Webb, 1940; Parry, 1960). Because it produces urine and is undoubtedly concerned with ionic regulation, there is a tendency to discuss antennal gland function in terms of the physiology of the vertebrate kidney. The unqualified inference, often expressed in general zoology texts, that the antennal gland carries out all the functions of the vertebrate kidney is unwarranted, since information concerning antennal gland function, other than ionic regulation, is extremely limited. This is particularly so when considering nitrogen excretion by the antennal gland. A urine-producing ‘excretory organ’ is usually thought of as being concerned with, amongst other things, the elimination of nitrogenous waste products. This function is sometimes ascribed to the antennal gland, despite the fact that there is remarkably little information on this aspect of the physiology of the organ. After a survey of nitrogen excretion by Crustacea and a consideration of analyses of the urine of Maia, Delaunay (1931) concluded that the antennal gland was not concerned with nitrogen excretion and that its importance in this respect has been over-estimated by histo-physiologists. With only a little additional information available, Parry (1960) concluded that the antennal gland was concerned primarily with ionic regulation. Ramsay (1961) was more cautious and considered that because information is so limited, and because little is known of invertebrate biochemistry, the possibility remains that the antennal gland may be concerned with the removal of some excretory products.

Because the excretion of nitrogen by Crustacea has been little studied, it is considered worthwhile to offer some data resulting from a study of antennal gland function in Carcinus. Measurements of the contribution of urine nitrogen to total nitrogen excretion by Crustacea have not been made. In this paper, figures obtained from analyses of some nitrogenous components of blood and urine of Carcinus are presented. Using this information, the role of the antennal gland of this animal in general nitrogen excretion can be estimated.

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Experimental procedure and methods of collecting blood and urine samples have been described earlier (Binns, 1969a).

Blood and urine were analysed for four components: ammonia, urea, uric acid and amino acid nitrogen.

Ammonia was estimated by the Conway microdiffusion method (Conway, 1950). Urea concentrations were determined by the same method, after first determining ammonia concentrations and then treating body-fluid samples with urease in a phosphate buffer solution.

α-Amino nitrogen was determined by the method of Folin (1922) as modified by Danielson (1933). Glycine standards were used for calibration and concentrations of amino acids in body fluids are expressed as equivalent to glycine concentrations. For ease of handling, proteins in blood were precipitated using 10% trichloracetic acid. It was not necessary to treat urine samples with the protein precipitant.

Uric acid was measured by the method of Benedict (1922) as described by Delory (1949).

For the colorimetric estimation of amino acids and uric acid a Biochem H 810 Absorptiometer, with appropriate filters, was used.

Table 1. Concentrations of some nitrogenous constituents in blood and urine of Carcinus

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Blood</th>
<th>Urine</th>
<th>Blood</th>
<th>Urine</th>
<th>Blood</th>
<th>Urine</th>
<th>Blood</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration, as mg. % (mean ± S.E.)</td>
<td>1.61 ± 0.29</td>
<td>1.16 ± 0.20</td>
<td>3.30 ± 1.25</td>
<td>0.82 ± 0.12</td>
<td>1.50 ± 0.12</td>
<td>0.82 ± 0.12</td>
<td>0.61 ± 0.33</td>
<td>0.64 ± 0.15</td>
</tr>
<tr>
<td>No. of observations</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>6</td>
<td>21</td>
<td>14</td>
<td>57</td>
<td>24</td>
</tr>
<tr>
<td>Concentration, as mg. N %</td>
<td>1.32</td>
<td>0.96</td>
<td>2.48</td>
<td>0.83</td>
<td>0.50</td>
<td>0.83</td>
<td>1.155</td>
<td>1.19</td>
</tr>
<tr>
<td>Delaunay (1931) (mg. N %)</td>
<td>2.10</td>
<td>—</td>
<td>2.70</td>
<td>—</td>
<td>0.40</td>
<td>—</td>
<td>12.0</td>
<td>—</td>
</tr>
</tbody>
</table>

Total urine nitrogen due to these four constituents = 3.25 mg. N/100 ml.

* Expressed as equivalent to glycine concentrations.

RESULTS

The results of the analyses of blood and urine from freshly captured crabs are given in Table 1. Figures obtained by Delaunay (1931) for Carcinus blood are also included for comparison.

These ‘normal’ values for nitrogenous constituents in the blood and urine of crabs direct from the field are generally of the same order as those found by Delaunay for Carcinus and for Maia.

A feature of these estimations is that the concentrations of presumed excretory nitrogenous compounds are greater in the blood than in the urine. Ammonia tends to be slightly higher in the blood than in the urine, the blood concentration of uric acid is almost double and the blood urea treble the respective urine concentrations of these molecules.
DISCUSSION

It now appears that the urine of *Carcinus* is produced by a so-called filtration process. Urine/blood (U/B) ratios of 1 for both inulin and sorbitol in crabs living in 100% sea water (Binns, 1969a) and a tendency for glucose U/B ratios to approach unity after treatment with phloridzin (Binns, 1969c) indicate that the primary urine is produced by a non-selective movement of a protein-free filtrate of the blood into the antennal gland.

In view of this it is perhaps surprising that the blood concentrations of uric acid and of highly diffusible, small molecules such as urea and ammonia are apparently greater than the respective urine concentrations of these substances. Comparable results have been found for another crustacean but have not been commented upon. Delaunay (1931) found that in *Maia* the recorded blood concentrations of ammonia and urea tended to be higher than those in the urine.

A possible explanation of these anomalies is that the procedures or analytical methods used gave erroneously high blood concentrations for uric acid, ammonia and urea. For instance, it is likely that Benedict's arsenophosphotungstic acid reagent is not entirely specific for uric acid. Another reactive material present in the blood but not in the urine (say, some large molecule not filtered into the antennal gland) would tend to give a high blood 'uric acid' concentration. The presence of bacteria or of naturally occurring enzymes in blood samples may have resulted in the production of additional ammonia and urea during the time blood samples were standing prior to being analysed. These discrepancies are pointed out, but they are taken to indicate technical difficulties rather than a real physiological difference between the concentrations of these nitrogenous compounds in blood and urine. More, carefully controlled, estimates would be needed to demonstrate conclusively that any of these familiar excretory products are, in fact, more concentrated in the blood than in the urine.

Large numbers of both marine and freshwater Crustacea have been shown to be predominantly ammonotelic (Delaunay, 1934; Dresel & Moyle, 1950), this method of eliminating waste nitrogen being associated with the aquatic habit. More than 80% of the total nitrogen excreted by *Carcinus* is in the form of ammonia (Needham, 1955). *Eriocheir* in distilled water loses ammonia at the same rate regardless of whether the openings of the antennal glands are blocked or unblocked (Krogh, 1938). Urine ammonia represents only 4–12% of the total nitrogen content of the urine of *Maia squinado*, and is not likely to be an important fraction of the total ammonia lost (Delaunay, 1931). Since the urine of *Carcinus* does not contain large amounts of ammonia, or of the other nitrogenous compounds estimated, and the antennal gland does not concentrate any of them, the possible importance of this organ in the excretion of nitrogen needs to be examined.

The contribution of the urinary nitrogen to general nitrogen excretion can be calculated from a knowledge of the urine concentrations of the four nitrogenous compounds estimated (this paper), taking the rate of urine production in normal sea water to be 4.35% body weight per day (Binns, 1969b) and the rate of nitrogen elimination by *Carcinus* to be 1.1 mg. N/25 g. crab/day (Needham, 1955). These calculations show that the elimination of nitrogen as ammonia, urea, uric acid and \(\alpha\)-amino nitrogen in the urine accounts for 3.2% of the total nitrogen excreted by
Carcinus. Ammonia, the animal’s main excretory product, enters the external medium almost entirely by diffusion. Only 1.04% of the total nitrogen loss can be accounted for by ammonia nitrogen in the urine. Nitrogen excreted by the kidney of *Salmo gairdneri*, a freshwater teleost, has been measured and it is interesting to note that, in this animal also, the excretory organ contributes only 3% of the total nitrogen loss (Fromm, 1963).

Because of the extremely small amounts of nitrogen eliminated in the urine of *Carcinus* the antennal gland must be regarded as being unimportant from the point of view of general nitrogen excretion.

Despite this conclusion, it is possible that the antennal gland may be concerned with the excretion of waste products from the blood. Total reducing substances (T.R.S.) equivalent to 14.5 mg. glucose % are present in the urine of *Carcinus* in 100% sea water, but the urine contains less than 1 mg. glucose % at normal blood glucose concentrations (Binns, 1969c). The reducing substances in the urine, other than glucose, have not been identified. It has been suggested that substances such as uric acid, ascorbic acid, amino sugars, tyrosine, dihydroxyphenylalanine and other phenols, or sulphhydryl compounds may all contribute to the T.R.S. value if they are present in body fluids (Wyatt, 1961). Secretory activity, indicated by the concentration of injected dyes, has been demonstrated in the antennal gland (Cuénot, 1893; Lison, 1942) and it is possible that complex, indiffusible molecules, which cannot otherwise be disposed of by metabolism, may be secreted and contribute to the relatively high concentration of T.R.S. in the urine. Until more is known about the composition of the urine, the question of whether the antennal gland is of importance in clearing any waste products of metabolism from the blood cannot be decided.

This work constitutes part of a Ph.D. thesis submitted to the University of Newcastle upon Tyne. It is a pleasure to thank Professor J. Shaw for his continual interest, and for invaluable help given during the course of the work. I am also grateful to S.R.C. for providing a postgraduate studentship.

**REFERENCES**


The antennal gland of Carcinus maenas (L.). V


KROGH, A. (1938). The active absorption of ions in some freshwater animals. Z. vergl. physiol. 25, 335-50.


