MICROPUNCTURE STUDIES OF THE CONCENTRATIONS OF SODIUM, POTASSIUM AND INULIN IN THE CRAYFISH ANTENNAL GLAND

BY J. A. RIEGEL

Zoology Department, Westfield College, University of London

(Received 21 August 1964)

INTRODUCTION

The evidence presently available supports a 'filtration'-reabsorption hypothesis for the mechanism underlying urine formation by the antennal gland of the crayfish. This evidence has been summarized most recently by Schmidt-Nielsen & Laws (1963) and by the writer (1963).

In a previous paper (Riegel, 1963) the results of measurements of chloride concentration and of osmotic pressure made on fluid removed from the antennal gland by micropuncture were presented. The present paper is concerned with an extension of micropuncture studies which ultimately are designed to yield as complete a picture as possible of the changes in water content and concentrations of electrolytes and organic molecules during the passage of urine through the antennal gland. The substances under consideration here are sodium, potassium and inulin.

MATERIALS AND METHODS

The crayfishes studied were specimens of Orconectes limosus (Rafinesque) and Austropotamobius pallipes pallipes (Lereboullet). The former species has been introduced into Western Europe from its native North America. The specimens studied were obtained from France.

The methods used for collecting and storing samples of blood and urine were the same as those reported earlier (Riegel, 1963). Samples for analyses of sodium and potassium were taken up in micropipettes of 16-150 ml. capacity and transferred to 5 ml. of deionized distilled water in polythene vessels. They were then analysed with a Unicam SP. 900 flame spectrophotometer.

It is worth commenting that polythene vessels, which were used for storing dilute solutions, take up ions from solution, but the uptake ceases for all practical purposes after the vessels have been in use for about 2 weeks.

The inulin used in this study was carboxy-labelled with 14C. Samples for inulin analysis were taken up in the same pipettes as were used for sodium and potassium. They were transferred to 1 ml. of a dioxane-based liquid scintillator (Nuclear Enterprises 220) and counted in an IDL counter.
RESULTS

In only two animals (O-3 and A-I in Tables 1 and 2) was it possible to confirm that samples were obtained from the proximal tubule. Such confirmation was made by dissecting the antennal gland after the micropuncturing procedure was completed and noting the presence or absence of blue liquid paraffin in the tubule. The samples listed in the tables under proximal tubule may have come from that portion of the antennal gland or from the most proximal portion of the distal tube.

Table 1. Sodium concentration (mM./l.) of blood and urine samples removed from various parts of crayfish antennal glands

(I. Specimens of Orconectes and II, specimens of Austropotamobius into which inulin was not injected. III, Specimens of Austropotamobius into which 14C-inulin was injected. Mean sodium concentration of the urine is significantly different \( P = < 0.01 \) from mean sodium concentration of the blood, except for the distal tubule of animals A-I to A-VII inclusive.)

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Blood</th>
<th>Coelomosaic</th>
<th>Labyrinth</th>
<th>Proximal tubule</th>
<th>Distal tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-1</td>
<td>242</td>
<td>176</td>
<td>160</td>
<td>121</td>
<td>152, 167</td>
</tr>
<tr>
<td>O-2</td>
<td>264</td>
<td>222</td>
<td>—</td>
<td>199</td>
<td>128, —</td>
</tr>
<tr>
<td>O-3</td>
<td>252</td>
<td>181</td>
<td>175</td>
<td>247</td>
<td>234, 177</td>
</tr>
<tr>
<td>O-4</td>
<td>265</td>
<td>202</td>
<td>—</td>
<td>172</td>
<td>162, 208</td>
</tr>
<tr>
<td>O-5</td>
<td>217</td>
<td>245</td>
<td>—</td>
<td>—</td>
<td>190, 217</td>
</tr>
<tr>
<td>O-6</td>
<td>215</td>
<td>208</td>
<td>—</td>
<td>123</td>
<td>220, 146</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>242 ± 20</td>
<td>207 ± 19</td>
<td>168</td>
<td>168 ± 42</td>
<td>181 ± 38, 183 ± 26</td>
</tr>
</tbody>
</table>

A-I       204   158      158       159      178        185
A-2       222   218      200       168      210        190
A-3       245   —        197       190      148        150
II A-4    252   200      212       200      162        170
A-5       230   195      200       —        222        183
A-6       252   195      200       192      167        172
| Mean ± S.D. | 234 ± 18 | 193 ± 20     | 194 ± 17  | 182 ± 16    | 181 ± 26, 175 ± 13 | 14.3 ± 3.7 |

A-I       226   217      155       —        —         —         26.0
A-II      228   193      190       150      —         —         26.0
A-III     192   185      —         —        190        285      25.0
III A-IV  218   170      190       157      157        375      16.5
A-V       208   179      167       167      —         —         27.0
A-VI      212   166      137       185      190        175      —
A-VII     —     195      —         175      192        198      27.5
| Mean ± S.D. | 214 ± 12 | 186 ± 16     | 168 ± 20  | 167 ± 12    | 182 ± 15, 219 ± 88 | 24.1 ± 3.8 |

The limits of error of the various analyses were as follows: sodium, ± 2% potassium, c. ± 10%, inulin, ± 5%. These estimates of accuracy were obtained by making practice runs with solutions of known concentrations. The estimate of error for potassium is less certain because of the relative insensitivity of the flame spectrophotometer for that element. This necessitated the use of prism slit widths at which considerable interference from other substances in the standards and experimental solutions was experienced. In later analyses (Table 2, animals A-2 to A-6) a pen recorder (Moseley ‘Autograph’ model 680) was used, which considerably extended the sensitivity of the flame spectrophotometer, permitting a much narrower prism slit to be used. There appeared to be no differences between results obtained with the recorder and those obtained with the galvanometer of the SP. 900.
Micropuncture studies of the crayfish antennal gland

The standards for the flame spectrophotometer were made up to concentrations covering the range of concentrations in the blood. Some elements may have been more concentrated in the urine than in the blood. Further, it is possible that interfering substances were present in the blood and urine. Nevertheless, the differences in potassium concentrations within the antennal gland were of such magnitude as to make it highly unlikely that they were due to analytical error.

Table 2. Potassium concentration (mM/l.) of blood and urine samples removed from various parts of crayfish antennal glands

(I, Specimens of Orconectes and II, specimens of Austropotamobius into which inulin was not injected. III, Specimens of Austropotamobius into which 14C-inulin was injected. Mean potassium concentration of the urine is significantly different (P < 0.05) from mean potassium concentration of the blood.)

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Blood</th>
<th>Coelomosac</th>
<th>Labyrinth</th>
<th>Proximal tubule</th>
<th>Distal tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-1</td>
<td>3.50</td>
<td>4.80</td>
<td>6.20</td>
<td>15.6</td>
<td>11.5</td>
</tr>
<tr>
<td>O-2</td>
<td>7.39</td>
<td>8.46</td>
<td>—</td>
<td>14.2</td>
<td>13.2</td>
</tr>
<tr>
<td>O-3</td>
<td>5.28</td>
<td>9.59</td>
<td>7.50</td>
<td>10.2</td>
<td>12.0</td>
</tr>
<tr>
<td>II-4</td>
<td>9.70</td>
<td>9.50</td>
<td>—</td>
<td>14.0</td>
<td>16.8</td>
</tr>
<tr>
<td>O-5</td>
<td>9.67</td>
<td>10.7</td>
<td>—</td>
<td>14.0</td>
<td>9.67</td>
</tr>
<tr>
<td>O-6</td>
<td>8.68</td>
<td>8.00</td>
<td>—</td>
<td>12.7</td>
<td>15.8</td>
</tr>
</tbody>
</table>

Mean ± s.d. 7.06 ± 2.09  8.51 ± 1.87  6.85 ± 1.33 ± 1.8  13.9 ± 1.9  11.2 ± 4.1  0.89 ± 0.46

A-1  5.98  13.9  16.2  — 21.1  11.4  —
A-2  6.45  17.7  12.9  14.6  13.2  19.8  0.72
A-3  6.45  —  17.7  12.9  12.9  22.2  13.5  1.08
IIA-4  7.64  18.2  12.7  13.4  12.0  14.8  0.84
A-5  3.60  15.2  13.8  — 15.6  14.1  2.57
A-6  9.24  12.7  14.1  14.8  13.0  18.1  2.90

Mean ± s.d. 6.56 ± 1.72  15.5 ± 2.1  14.6 ± 1.8  13.9 ± 0.8  16.2 ± 4.0  15.3 ± 2.8  1.64 ± 0.92

A-I  8.42  17.7  20.6  —  —  —  0.52
A-II  7.07  16.4  17.7  24.0  —  —  0.52
A-III  8.50  14.2  —  —  16.5  18.5  1.75
IIA-IV  6.50  12.5  21.2  23.2  15.7  —  0.55
A-V  5.50  9.20  10.0  25.2  —  —  1.20
A-VI  5.05  11.8  27.1  30.8  10.2  23.8  —
A-VII  11.8  18.5  26.8  20.0  16.5  0.20

Mean ± s.d. 6.84 ± 1.32  13.3 ± 2.7  19.2 ± 5.1  26.0 ± 2.7  15.6 ± 3.5  19.6 ± 3.1  0.96 ± 0.47

In Tables 1 and 2 are presented the results of measurements of sodium and potassium in the antennal glands of nineteen crayfishes. Included are seven specimens of Austropotamobius into which inulin was injected (animals A-I to A-VII) and on which the concentrations of inulin, potassium and sodium were determined for both blood and urine. The blood and urine were analysed for sodium and potassium only in six specimens of Orconectes (animals O-1 to O-6, Tables 1 and 2) and six specimens of Austropotamobius (animals A-I to A-6, Tables 1 and 2). The species are listed separately in the tables because there were differences between the two species in the mean concentrations of sodium and potassium.

The concentrations of sodium in the blood and in the urine, especially in the urine from the coelomosac and labyrinth, were different in the two lots of Austropotamobius. The concentrations of sodium in the crayfishes which had been injected with inulin...
were higher than in the uninjected animals. The reason for this is not known. The sodium analyses of the crayfishes which had been injected with inulin were made in the winter and early spring. The analyses of uninjected animals were made in the summer. Thus there may have a normal winter-summer difference in the level of blood sodium. However, urine: blood (U/B) ratios for sodium and potassium were the same for both lots of crayfishes.

In Table 3 are presented the U/B ratios for inulin. The concentration of inulin in the blood lay within the range of 12.6-46.8 mg. %.

Table 3. Urine: blood ratios for inulin of urine samples removed from various parts of crayfish antennal glands

(All means are significantly different from one (P = < 0.05), except the mean of the U/B ratios for inulin in the bladder. The P values listed under the mean U/B ratios for inulin in the various parts of the antennal gland indicate the significance of the difference of the mean U/B ratio for inulin in the coelomosac and the mean U/B ratios for inulin in the various other parts of the antennal gland.)

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Coelomosac</th>
<th>Labyrinth</th>
<th>Proximal tubule</th>
<th>Distal tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-I</td>
<td>1.29</td>
<td>1.46</td>
<td>1.65</td>
<td>—</td>
</tr>
<tr>
<td>A-II</td>
<td>1.07</td>
<td>1.41</td>
<td>1.85</td>
<td>—</td>
</tr>
<tr>
<td>A-III</td>
<td>1.12</td>
<td>1.50</td>
<td>1.40</td>
<td>2.93</td>
</tr>
<tr>
<td>A-IV</td>
<td>1.13</td>
<td>1.31</td>
<td>1.20</td>
<td>2.16</td>
</tr>
<tr>
<td>A-V</td>
<td>1.42</td>
<td>1.54</td>
<td>1.33</td>
<td>—</td>
</tr>
<tr>
<td>A-VI</td>
<td>0.87</td>
<td>0.74</td>
<td>1.11</td>
<td>1.20</td>
</tr>
<tr>
<td>A-VII</td>
<td>1.20</td>
<td>1.49</td>
<td>1.86</td>
<td>1.69</td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td>1.16±0.16</td>
<td>1.35±0.26</td>
<td>1.49±0.28</td>
<td>2.12±0.96</td>
</tr>
</tbody>
</table>

From Table 1 it may be seen that the sodium concentrations in the coelomosac, labyrinth and proximal tubule are significantly below that in the blood. In the distal tubule, however, the sodium concentration rises. In the crayfishes into which inulin had been injected, the sodium concentration in the distal tubule is, in some cases, greatly in excess of that in the blood.

The potassium concentrations in the antennal gland are consistently greater than in the blood in all parts of that organ except the bladder.

U/B ratios for inulin in the various parts of the antennal gland are greater than one, except in the bladder and in one instance (animal A-VI, Table 3) in the labyrinth and coelomosac. As between the various parts of the antennal gland, statistically significant differences (P = < 0.10) were found between the mean U/B ratio for inulin of the coelomosac and the mean U/B ratios for inulin of the labyrinth, the proximal tubule and the distal tubule. The differences of the mean U/B ratios for inulin between the coelomosac and bladder and between the parts of the antennal gland lying distal to the coelomosac were not statistically significant.
DISCUSSION

The results of the studies reported here do not provide unequivocal evidence for the mechanism of primary urine formation in the crayfish antennal gland. However, they are wholly consistent with a ‘filtration’ hypothesis. With the recent finding of a filtration site in the coelomosac of the crayfish antennal gland (Kümmel, 1964, and personal communication), it seems no longer necessary to consider the evidence for arterial filtration inconclusive. Whether or not arterial pressures within the coelomosac are sufficiently high to effect filtration can only be judged indirectly. As pointed out by the writer in 1963, the fact that the blood circulates within the non-rigid antennal gland against the general haemocoelic pressure indicates that arterial pressures in the antennal gland exceed the hydrostatic pressures of the haemocoel and the urine. Since the colloid osmotic pressure of the blood exceeds that of the urine (Picken, 1936), filtration under the influence of arterial hydrostatic pressure is indeed likely.

The high concentrations of potassium in the coelomosac and labyrinth may indicate that secretory processes play a large part in determining the composition of the urine in those parts. However, the present results do not lend support to any particular mechanism out of the many which might be suggested. Therefore, it would seem to be fruitless to speculate at this stage of these investigations.

The sodium concentrations in the coelomosac and labyrinth indicate that that ion is reabsorbed there. In the coelomosac, where the urine is isosmotic to the blood (Riegel, 1963), the sodium absorption may or may not be active; but in the labyrinth, where the urine is hypo-osmotic to the blood, sodium reabsorption is probably active.

The high concentration of sodium in the tubule is surprising. The sodium concentration is more than double the chloride concentration reported earlier (Riegel, 1963). The average osmotic pressure in the distal portion of the distal tubule was found to be equivalent to 121 mm./l. NaCl (op. cit.). The reasons for this discrepancy are unknown.

Grobben (1880) was able to relate the length of the tubular portions of the nephridia of various animals to the habitat in which they live. He found that the tubular portion was longest in freshwater or terrestrial animals. Schlieper & Herrmann (1930) concluded that the long tubular portion of the crayfish antennal gland is responsible for producing the hypotonic urine of that animal. However, this conclusion is not borne out by recent studies.

Kamemoto, Keister & Spalding (1962) and Riegel (1963) have suggested that the bladder plays a role in diluting the urine. This suggestion is supported further by the present studies. Even in the most distal portions of the tubule the sodium and potassium concentrations are not statistically different from those in the coelomosac. Yet, in the fully distended bladder, the concentrations of those ions are always low. Further, as indicated by the U/B ratios for inulin, the amount of water in the urine decreases in the coelomosac and labyrinth. It remains low (relative to the blood) in the tubular portions of the antennal gland.

In one instance (animal A-5), the writer was able to collect a sample of urine from the place where the bladder and distal tubule join. This normally is not possible because the bladder is destroyed and its contents contaminated with blood when the
antennal gland is removed from the crayfish for micropuncture. The sodium and potassium concentrations in the sample obtained from the junction of the bladder and distal tubule were 112 and 33.1 mM./l, respectively. Thus it seems probable that the bladder plays a very important role in diluting the urine: a role, which on presently available evidence would appear to overshadow the role of the tubule.

**SUMMARY**

1. The concentrations of sodium, potassium and inulin in the blood and urine have been measured at various sites in the antennal gland of *Orconectes limosus* and *Austro-potamobius pallipes pallipes*.

2. The sodium concentration in all parts of the antennal gland is less than the sodium concentration in the blood. Only in the bladder is the sodium concentration significantly different from the sodium concentration in the other parts.

3. The potassium concentration in all parts of the antennal gland, except the bladder, is greater than the potassium concentration in the blood. The potassium concentration within the bladder is much less than that in the blood.

4. The U/B ratio for inulin exceeds one in all parts of the antennal gland.

5. These results are discussed in relation to the function of various parts of the antennal gland and to the manner in which urine is formed by the crayfish antennal gland.

It is a pleasure to acknowledge the Medical Research Council for their generosity in supporting this work by providing me with an expenses grant.

**REFERENCES**


