STUDIES ON THE RENAL EXCRETION OF ELECTROLYTES BY THE TROUT
(SALMO GAIRDNERI)*

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INTRODUCTION

Many species of teleosts within the family Salmonidae are anadromous. Having hatched in fresh water, these fish undergo certain morphological changes prior to commencing their seaward migration. This period of morphological and behavioural change is known as smolting and is characterized by the deposition of guanine in the ventral and ventro-lateral dermal layer (Hitchings & Falco, 1944; Sumner, 1944; Neckel, 1954), an alteration in the body proportions (Hoar, 1939) and the development of a preference for an environment of increased salinity (Baggerman, 1960). These morphological and behavioural changes are in turn accompanied by, or are due to, certain physiological adjustments. For instance, along with the development of salinity preference, smolting Salmo salar show a more rapid regulation of their tissue electrolyte concentrations after abrupt transfer to sea water (Houston, 1959; Parry, 1960). These regulatory processes may be in part associated with the increases in adrenocortical volume (Olivereau, 1962) and circulating concentrations of 17-hydroxycorticosteroids (Fontaine & Hatey, 1954) at the time of smolting. Certainly the administration of adrenocortical steroids to saline-loaded rainbow trout, S. gairdneri, and intact brown trout, S. trutta, is accompanied by an increased extra-renal excretion of sodium (Holmes, 1959) and a reduction in plasma sodium concentrations (Chester Jones, 1956; Holmes & Butler, 1963).

In order to maintain homeostasis during and after adaptation to a marine environment it is necessary that, among other things, changes occur in the water and electrolyte regulatory processes of the fish. These changes involve the development of an extra-renal pathway for the excretion of electrolytes and a considerable modification in the pattern of renal excretion of water and electrolytes. We were diverted from our studies of the effects of various hormones on renal functions in S. gairdneri by the observation that changes in the renal excretory pattern were occurring in untreated fish during the period of parr-smolt transformation. The changes which occurred in the renal excretory processes of a stock of steelhead trout (S. gairdneri) during the pre-smolting condition, through the period of smolting and into the post-smolting condition are the substance of this report.

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MATERIALS AND METHODS

Hatchery-raised *Salmo gairdneri* were maintained in running de-chlorinated tapwater (Na = 0.022 m-equiv./l., K = 0.0042 m-equiv./l. Cl = 0.024 m-equiv./l.) at seasonal temperatures and photoperiods.

Experimental fish were starved for one week prior to anaesthesia with MS 222 (Tricainmethane sulfonate). The flared end of a polyethylene cannula (Becton-Dickinson PE60) was inserted into the urino-genital papilla and secured by two ligatures. The first ligature was fastened around the papilla and adjusted until patent. The cannula was then firmly held in place by a purse-string ligature through the body wall anterior to the papilla and tied posteriorly.

Fig. 1. An exploded diagram of the tank used for housing trout during the period of urine collection. The tank was constructed from heavy Plexiglass and was adjustable to the length, height and girth of the fish. The tank was adjusted for girth by fitting the partition C into the appropriate grooves in the end-plates G and H. The compartment was adjusted to the length of the fish by slotting the perforated plate F into one of a pair of grooves in partition C and the front of the box, D. The height of the box was adjusted by means of the four screws in the lid A; these screws supported the lid by sitting on the longitudinal ledges which ran along the inside of the sides B and D. All stippled pieces were made from opaque plastic and E could be removed for observation of the fish. Water entered the tank at the lower edge of G and emerged at the upper edge of H. The cannula was passed through a large hole at the base of H.

After cannulation the fish was placed in a tank which was adjusted to the length, breadth and depth of the individual (Fig. 1). The cannula was then passed through a hole of considerably larger diameter at the rear of the tank and attached to a vented
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collecting cylinder (Fig. 2). The long cannula permitted some freedom of movement by the fish without displacing the cannula from the urino-genital papilla. During the period of urine collection the fish were kept in the dark and no stress on the part of the fish was evident.

Pre-smolting fish were examined in the late summer of 1963, members of the same stock were studied in the spring of 1964 during the period of smolting and in the summer of 1964 in their post-smolting condition. In each group of smolting and non-smolting trout, urine outputs from intact fish and from fish injected with isotonic saline were examined. The isotonic saline was injected intraperitoneally at the time of cannulation and these fish served as controls for the separate series of trout used to determine glomerular filtration rates.

Fig. a. A section through the tank illustrated in Fig. 1 containing a cannulated fish with the cannula attached to the vented cylinder.

The fish were allowed to remain in the tanks for one hour after cannulation before the collection of urine was commenced. The urine was then collected for at least 20 hr. and the cumulative output was periodically recorded. At autopsy the fish were dried, weighed and terminal blood was collected by severing the tail. The blood samples were immediately centrifuged at 8000 rev./min. at 5°C. for 10 min. All urine and plasma samples were analysed for sodium, potassium, chloride and total osmolality. Sodium and potassium concentrations were measured by flame-photometry (Zeiss PF 5), chloride concentrations were determined by titration with silver ions (Cotlove automatic titrator) and osmolalities were estimated by the freezing-point technique using a Fiske osmometer. All urine flow rates and rates of excretion of ions were expressed in ml., m-equiv., or m-osmoles/kg. body weight/day.

Glomerular filtration rates were measured by the inulin clearance method. A single intraperitoneal dose of 25 mg. inulin in 0.5 ml. isotonic saline (NaCl, 0.78 %) was administered at the time of cannulation and urine collection was started 5 hr. later. At this time the plasma inulin concentrations were constant and remained so for the
remaining 8 hr. urine collection period. Terminal blood samples were collected in heparinized tubes and immediately centrifuged. Protein was precipitated from the plasma samples with cadmium sulphate (Fujita & Iwatake, 1931, as modified by Smith et al. 1945) and the supernatant was filtered through washed cotton.

Duplicate 2 ml. samples of plasma filtrate and urine were analysed for inulin according to the Schreiner (1950) modification of the direct resorcinol method of Roe, Epstein & Goldstein (1949). Standard solutions of inulin were included with each determination. From the inulin concentrations of plasma and urine and the urine flow rate the glomerular filtration was calculated as follows: rate of plasma filtration \( (C_f) = u \cdot v / p \) ml./min. where \( u = \) urine inulin concentration (mg./ml.), \( v = \) urine flow rate (ml./min.) and \( p = \) plasma inulin concentration (mg./ml.). The glomerular filtration rate (G.F.R.) for each fish was then expressed in ml./kg. body wt./day.

All mean values were expressed ± the standard error of the mean (S.E.M.) and mean values were statistically compared according to the Student 't' test, (Snedecor, 1956). All regressions were estimated by the method of least squares and compared by the analysis of covariance (Snedecor, 1956).

**RESULTS**

**Intact fish**

In both the smolting and the non-smolting fish the rate of urine flow during the collection period was always linear with respect to time. When smolting began there was a very significant decrease in the rate of urine flow and this lower rate was sustained throughout the smolting period (Table 1). During the period of transformation from the smolting to the post-smolting condition there was a restoration of the pre-smolting urine flow rates (Table 1). Although these flow rates were considerably more variable than the values recorded prior to smolting they did not differ significantly from these values. It is likely that the post-smolting fish were examined

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of fish</th>
<th>Body wt. (g.)</th>
<th>Regression* ( Y = a + bX )</th>
<th>Degrees of freedom</th>
<th>'F' value</th>
<th>'P' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-smolt</td>
<td>10</td>
<td>169.3 ± 4.4</td>
<td>( Y = 2.22 + 0.18X )</td>
<td>1,198</td>
<td>109.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Smolt</td>
<td>14</td>
<td>174.7 ± 9.2</td>
<td>( Y = 0.98 + 0.57X )</td>
<td>1,138</td>
<td>0.27</td>
<td>&gt; 0.1</td>
</tr>
<tr>
<td>Post-smolt</td>
<td>8</td>
<td>163.2 ± 8.9</td>
<td>( Y = 0.69 + 0.85X )</td>
<td>1,138</td>
<td>0.27</td>
<td>&gt; 0.1</td>
</tr>
</tbody>
</table>

* \( Y = a + bX \), where \( Y = \) cumulative urine flow (ml./kg. body wt.), \( b = \) rate of urine flow (ml./kg. body wt./hr.), \( X = \) time in hours, and \( a = \) the ordinate intercept.
† \( S_y = \) standard deviation from the regression.
‡ \( S_b = \) standard error of the regression coefficient (\( b \)).
§ \( r = \) correlation coefficient.

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**Table 1. The rate of urine flow from intact smolting and non-smolting trout** (Salmo gairdneri) **maintained in running fresh water at seasonal temperatures and photoperiods**

(The urino-genital papilla of each fish was cannulated 1 hr before the commencement of urine collection and urine was collected for at least 20 hr.)
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Closer to the period of transformation than were the pre-smolting trout. Consequently, more fish were sampled in which the higher urine flow rates had not been completely restored in spite of the fact that these fish showed no overt symptoms of smolting.

Although the mean values were consistently higher, the urine electrolyte concentrations of the smolting fish did not differ significantly from the pre-smolting values. The total osmolal and potassium concentrations of the urine from post-smolting fish were, however, significantly lower than the corresponding values for the smolts (Table 2). Possibly these observations reflect an 'overshoot' in the regulatory mechanisms responsible for the restoration of the pre-smolt pattern of renal excretion.

Table 2. The urine electrolyte concentrations from intact smolting and non-smolting trout (Salmo gairdneri) maintained in fresh water at seasonal temperatures and photoperiods

(The urino-genital papilla of each fish was cannulated 1 hr. before the commencement of urine collection and urine was collected for at least 20 hr. All values are expressed as means ± S.E.M. The number of fish and their body weights are the same as those reported in Table 1.)

<table>
<thead>
<tr>
<th></th>
<th>Urine concentration (m-equiv. or m-osm./L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>Pre-smolt</td>
<td>8.96±1.29</td>
</tr>
<tr>
<td>Smolt</td>
<td>12.08±1.46</td>
</tr>
<tr>
<td>Post-smolt</td>
<td>11.68±2.24</td>
</tr>
</tbody>
</table>

Significance with respect to the corresponding smolt value: *P < 0.05, **P < 0.01, ***P < 0.001.

Table 3. The excretion of water and electrolytes by intact smolting and non-smolting trout (Salmo gairdneri) maintained in running fresh water at seasonal temperatures and photoperiods

(The urinogenital papilla of each fish was cannulated 1 hr. before the commencement of urine collection and urine was collected for at least 20 hr. All values are expressed as the mean ± S.E.M.)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of fish</th>
<th>Body weight (g.)</th>
<th>Total renal excretion (ml., m-equiv. or osm./L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>Pre-smolt</td>
<td>10</td>
<td>169.3</td>
<td>115.9***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±4.4</td>
<td>±8.3</td>
</tr>
<tr>
<td>Smolt</td>
<td>9</td>
<td>179.0</td>
<td>51.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±10.6</td>
<td>±3.9</td>
</tr>
<tr>
<td>Post-smolt</td>
<td>8</td>
<td>163.2</td>
<td>112.8***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±8.9</td>
<td>±4.4</td>
</tr>
</tbody>
</table>

Significance with respect to the corresponding smolt value: *P < 0.05, **P < 0.01, ***P < 0.001.

With the exception of chloride, the total daily excretion of water and electrolytes by the smolting fish was significantly lower than in the pre-smolting fish and these lower excretory rates were restored to the pre-smolting levels in the post-smolting fish (Table 3).

The discrepancies between the numbers of fish reported in Table 1 and Tables 2 and 3 are due to the fact that some fish dislodged their cannulae and therefore urine was not collected from these fish for the full 20 hr. period.
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Injected fish

In order to determine the glomerular filtration rates of the various groups of fish it was necessary to inject some individuals with inulin. The renal excretory patterns of smolting and non-smolting trout which had received a single intraperitoneal injection of isotonic saline were therefore used as controls for the fish in which inulin clearance determinations were made.

Table 4. The rate of urine flow from isotonic-saline injected smolting and non-smolting trout (Salmo gairdneri) maintained in running fresh water at seasonal temperatures and photoperiods

(Each fish received a single intraperitoneal injection of isotonic saline at the time of cannulation. Urine collection was started 1 hr. after cannulation and was continued for at least 20 hr.)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of fish</th>
<th>Body weight (g.)</th>
<th>Regression* Y = a + bX</th>
<th>Degrees of freedom</th>
<th>'F' value</th>
<th>'P' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-smolt</td>
<td>10</td>
<td>185.8 ± 11.2</td>
<td>Y = 2.3 + 0.12</td>
<td>1,172</td>
<td>136.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Smolt</td>
<td>12</td>
<td>174.5 ± 7.9</td>
<td>Y = 3.6 + 0.12</td>
<td>1,172</td>
<td>136.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Post-smolt</td>
<td>9</td>
<td>177.0 ± 11.4</td>
<td>Y = 0.10 + 0.21</td>
<td>1,125</td>
<td>1.62</td>
<td>&gt; 0.1</td>
</tr>
</tbody>
</table>

* Y = a + bX, where Y = cumulative urine flow (ml./kg body wt.), b = rate of urine flow (ml./kg body weight/hr.), X = time in hours, and a = the ordinate intercept.

† $s_a = $ standard deviation from the regression.

‡ $s_b = $ standard error of the regression coefficient (b).

§ $r = $ correlation coefficient.

Comparison of the urine flow rates of corresponding groups of intact and saline-injected fish did not show any consistent variation (cf. Tables 1 and 4). The mean urine flow rates of the intact pre-smolting and the smolting trout were higher than those of the corresponding saline-injected trout ($P < 0.05$). On the other hand, the urine flow rates of the two groups of post-smolting trout did not differ at all ($P > 0.7$).

In the saline-injected fish, however, the same changes in the patterns of urine flow that were observed in the intact fish were apparent during the transformation into and out of the smolting condition. Thus, when compared to the pre-smolting fish, a significantly lower urine flow rate occurred in the smolting trout. During the transition from the smolting to the post-smolting condition the higher urine flow rate was restored (Table 4).

As in the case of the intact fish the mean urine electrolyte concentrations were higher in the smolting than in the non-smolting groups of fish; only the sodium and chloride concentrations in the pre-smolting and the potassium concentration in the post-smolting trout, however, were significantly different from the corresponding values for the smolting fish (Table 5). Examination of ion concentration ratios for urine and plasma showed no evidence for any selective ion concentrating mechanism in the renal tubules of either the smolting or the non-smolting trout (Table 5).

It is of interest to note that the collective concentrations of the ion species measured
in the smolting and non-smolting trout urine would only account for approximately 50% of the observed urine osmolality. The total urine nitrogen concentrations in separate groups of ten smolting and ten non-smolting trout, *S. gairdneri*, starved for one week prior to urine collection, were $15.8 \pm 1.6$ and $8.4 \pm 0.4$ mg./100 ml. respectively and the corresponding plasma non-protein nitrogen concentrations were $42.5 \pm 1.3$ and $28.1 \pm 2.9$ mg./ml. plasma (Holmes & Stewart, unpublished). This

Table 5. The urine (*U*) and plasma (*P*) electrolyte concentrations and the urine: plasma (*U/P*) concentration ratios from saline-injected trout (*Salmo gairdneri*) maintained in fresh water at seasonal temperatures and photoperiods

(Each fish received a single intraperitoneal injection of isotonic saline at the time of cannulation. Urine collection was started 1 hr. after cannulation and was continued for at least 20 hr. All values are expressed as means ± s.e.m. The number of fish and their body weights are the same as those reported in Table 6.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sodium U (m-equiv. or m-osm./l.)</th>
<th>Sodium P</th>
<th>Potassium U (m-equiv. or m-osm./l.)</th>
<th>Potassium P</th>
<th>Chloride U (m-equiv. or m-osm./l.)</th>
<th>Chloride P</th>
<th>Osmolals U (m-osm./l.)</th>
<th>Osmolals P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-smolt</td>
<td>797 ± 0.97</td>
<td>133.9</td>
<td>1.40 ± 0.56</td>
<td>1.66</td>
<td>7.40 ± 0.4</td>
<td>118.1</td>
<td>34.1 ± 5.0</td>
<td>286.0</td>
</tr>
<tr>
<td></td>
<td>(U/P = 0.62 ± 0.02)</td>
<td></td>
<td>(U/P = 1.49 ± 0.45)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smolt</td>
<td>15.85 ± 2.76</td>
<td>135.2</td>
<td>1.97 ± 0.31</td>
<td>1.60</td>
<td>11.34 ± 1.46</td>
<td>123.9</td>
<td>59.7 ± 1.8</td>
<td>293.1</td>
</tr>
<tr>
<td></td>
<td>(U/P = 0.094 ± 0.002)</td>
<td></td>
<td>(U/P = 1.45 ± 0.31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-smolt</td>
<td>10.89 ± 1.10</td>
<td>131.0</td>
<td>1.28 ± 0.11</td>
<td>0.87</td>
<td>8.11 ± 0.30</td>
<td>124.7</td>
<td>35.9 ± 2.3</td>
<td>290.4</td>
</tr>
<tr>
<td></td>
<td>(U/P = 0.083 ± 0.01)</td>
<td></td>
<td>(U/P = 1.62 ± 0.13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance with respect to the corresponding smolt value: *P < 0.05.

value for the total urine nitrogen concentration of non-smolting trout is somewhat higher than an earlier reported value for this species when 60% of the nitrogen was in the form of ammonia and 40% in the form of urea (Fromm, 1963). Assuming a similar ammonia:urea ratio in the present study, then solutions containing the sodium, potassium and chloride corresponding to the concentrations reported in Tables 3 and 5, plus ammonia and urea equivalent to the above total urine nitrogen concentrations, gave real osmolalities of 48 and 24 m-osm./l. for smolting and non-smolting trout respectively. These values more closely approximate to the observed real osmolalities of these urine samples and strongly suggest that changes in the tubular secretion of nitrogenous compounds significantly influenced the total osmotic pressure of the excreted urines.

The mean values for the total daily excretion of water and electrolytes by the saline-injected pre-smolting fish were consistently lower than the corresponding values for the intact fish. Only in the case of water and potassium excretion, however, did these values approach significance (*P < 0.05*, cf. Tables 2 and 6). No significant differences could be detected between the total daily excretion of water and electrolytes by intact smolting and post-smolting fish and the corresponding groups which had received an injection of isotonic saline.
Upon entering the smolting condition there was a significant decline in the total daily excretion of water and potassium. The total osmolal, sodium and chloride excretion was not reduced significantly. In the post-smolting fish, however, the total daily excretion of water and all electrolytes measured was significantly higher than in the smolting fish (Table 6).

Table 6. The excretion of water and electrolytes for saline-injected smolting and non-smolting trout (Salmo gairdneri) maintained at seasonal temperatures and photoperiods

(Each fish received a single intraperitoneal injection of isotonic saline at the time of cannulation. Urine collection was started 1 hr. after cannulation and was continued for at least 20 hr. All values are expressed as means ± S.E.M.)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of fish</th>
<th>Body wt. (g.)</th>
<th>Total water excretion (ml., m-equiv. or m-osm./l.)</th>
<th>Total Na excretion (m-equiv. or m-osm./l.)</th>
<th>Total K excretion (m-equiv. or m-osm./l.)</th>
<th>Total Cl excretion (m-equiv. or m-osm./l.)</th>
<th>Total osmolal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-smolt</td>
<td>10</td>
<td>185.8 ± 11.2</td>
<td>94.5 ± 4.9 **</td>
<td>0.77 ± 0.12 **</td>
<td>0.14 ± 0.03 **</td>
<td>0.72 ± 0.13 **</td>
<td>3.26 ± 0.77 **</td>
</tr>
<tr>
<td>Smolt</td>
<td>10</td>
<td>174.4 ± 9.3</td>
<td>50.0 ± 3.8</td>
<td>0.58 ± 0.08</td>
<td>0.07 ± 0.01</td>
<td>0.54 ± 0.08</td>
<td>2.04 ± 0.15</td>
</tr>
<tr>
<td>Post-smolt</td>
<td>9</td>
<td>177.0 ± 11.4</td>
<td>115.9 ± 8.9</td>
<td>1.23 ± 0.15</td>
<td>0.14 ± 0.02</td>
<td>0.88 ± 0.14</td>
<td>4.15 ± 0.29</td>
</tr>
</tbody>
</table>

Significance with respect to the corresponding smolt value: *P < 0.05, **P < 0.01, ***P < 0.001.

Table 7. The glomerular filtration (inular clearance rates) of smolting and non-smolting trout (Salmo gairdneri) maintained in fresh water at seasonal temperatures and photoperiods

(Each fish received an intraperitoneal injection of 25 mg. inulin in 0.5 ml. isotonic saline at the time of cannulation. Urine collection was started 5 hr. after cannulation and continued for 8 hr. All means are expressed ± S.E.M.)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of fish</th>
<th>Body wt. (g.)</th>
<th>Glomerular filtration rate (ml./kg. body wt./day)</th>
<th>'P' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-smolt</td>
<td>10</td>
<td>176.7 ± 14.0</td>
<td>174.7 ± 9.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Smolt</td>
<td>9</td>
<td>166.0 ± 12.3</td>
<td>90.5 ± 7.8</td>
<td>—</td>
</tr>
<tr>
<td>Post-smolt</td>
<td>9</td>
<td>161.7 ± 27.6</td>
<td>181.9 ± 22.5</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Inulin clearance studies in separate groups of fish indicated that a significant decline in the glomerular filtration rate occurred in the smolting fish when compared to fish in the pre-smolting condition. The higher filtration rate was restored in the post-smolting trout (Table 7).

Assuming that the filtrate appearing in the Bowman’s capsule was an ultrafiltrate of the plasma, then, at equilibrium, the concentrations of the electrolytes in the plasma and the glomerular filtrate would be distributed according to the Gibbs–Donnan equilibrium. For an ultra-filtrate containing less than 0.5% protein the following factors \( k \) may apply: \( \frac{Na}{Na_p} \) and \( \frac{K}{K_p} = 0.94 \) and \( \frac{Cl}{Cl_p} = 1.02 \) where \( f \) and \( p \) represent the filtrate and plasma-ion concentrations in m-equiv./ml. respectively. From the glomerular filtration rate and the plasma ion concentrations the filtered load of each plasma component may therefore be calculated as follows: filtered load = \( kP_pC_f \), where \( k \) is the Gibbs–Donnan factor, \( P_p \) is the plasma ion concentration in m-equiv./ml. and \( C_f \) is the inulin clearance rate (G.F.R.) in ml. plasma/kg. body
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The filtered loads of sodium, potassium, chloride and total osmolals were thus derived from the plasma ion concentrations and the G.F.R. and are represented in Table 8. The filtered loads of all the plasma components measured were significantly lower in the smolting than in the pre-smolting fish. With exception of potassium, where the value remained similar to that observed in the smolts, the filtered loads of these components were restored to the pre-smolting levels in the post-smolting fish.

Table 8. The filtered load of sodium, potassium, chloride and osmolals by the smolting and non-smolting trout (Salmo gairdneri) maintained in running fresh water at seasonal temperatures and photoperiods

(All values are expressed as means ± S.E.M. The number of fish and their body weights are the same as those reported in Table 6.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Na</th>
<th>K</th>
<th>Cl</th>
<th>Osmolal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-smolt</td>
<td>22.0***±0.92</td>
<td>0.27**±0.05</td>
<td>21.0***±0.98</td>
<td>49.9***±0.90</td>
</tr>
<tr>
<td>Smolt</td>
<td>11.5 ±0.34</td>
<td>0.14 ±0.03</td>
<td>11.4 ±0.33</td>
<td>26.1 ±0.40</td>
</tr>
<tr>
<td>Post-smolt</td>
<td>22.4***±0.67</td>
<td>0.15 ±0.03</td>
<td>23.1***±0.43</td>
<td>52.8***±0.58</td>
</tr>
</tbody>
</table>

* Filtered load = kPmCf, where k is the correction factor for the Gibbs-Donnan equilibrium between the plasma and the glomerular filtrate, Pm = the plasma ion concentration (m-equiv./ml.) and Cf = the inulin clearance rate (G.F.R.) in ml/kg. body wt./day.

Significance with respect to the corresponding smolt value: *P < 0.05, **P < 0.01, ***P < 0.001.

Table 9. The percentage reabsorption of water, sodium, chloride and osmoles and the percentage secretion of potassium from the filtered loads of smolting and non-smolting trout (Salmo gairdneri) maintained in fresh water at seasonal temperatures and photoperiods

(All values are expressed as means ± S.E.M. The number of fish and their body weights are the same as those reported in Table 6.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Water</th>
<th>Na</th>
<th>K</th>
<th>Cl</th>
<th>Osmolal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-smolt</td>
<td>45.9 ±2.8</td>
<td>96.4 ±0.6</td>
<td>93.6 ±30.2</td>
<td>96.9 ±0.5</td>
<td>93.2 ±1.9</td>
</tr>
<tr>
<td>Smolt</td>
<td>44.6 ±1.3</td>
<td>95.0 ±0.7</td>
<td>92.9 ±26.8</td>
<td>95.3 ±0.7</td>
<td>92.3 ±0.6</td>
</tr>
<tr>
<td>Post-smolt</td>
<td>36.3 ±4.9</td>
<td>94.4 ±0.7</td>
<td>96.0 ±8.6</td>
<td>96.2 ±0.6</td>
<td>92.1 ±0.6</td>
</tr>
</tbody>
</table>

* Percentage reabsorption of water = [(1−(v/Cf))100, where v = urine flow rate (ml/kg. body wt./day) and Cf = inulin clearance (ml/kg. body wt./day). Percentage reabsorption of sodium, chloride and osmoles = [(1−(Uxv)/k PmCf)]100 where Ux = urine concentration (m-equiv./ml. or m-osm./ml.).

† Percentage secretion of potassium = [(Ux Pm)/k PmCf]100, where Uk and Pk = urine and plasma potassium concentrations (m-equiv./ml.) respectively.

The tubular reabsorption of water (T_{H2O}) was derived from the difference between the rate of inulin clearance (Cf) and the rate of urine flow (v) in ml/kg. body wt./day, so that T_{H2O} = Cf−v. Therefore, the percentage reabsorption of filtered water by the renal tubule = [(1−(v/Cf))100. Similarly, the tubular reabsorption of the filtered plasma components was estimated as follows: T_{x} = kP_{x}Cf−(U_{x}v), where T_{x} is the quantity reabsorbed (m-equiv. or m-osm./kg. body wt./day), kP_{x}Cf is the filtered load as defined above, U_{x} is the urine concentration (m-equiv. or m-osm./ml.) and v is the urine flow rate (ml/kg. body wt./day). The percentage reabsorption of the
filtered plasma components by the renal tubules was then calculated from the following equation: percentage reabsorption = \(\frac{1 - (U_x v)}{k P_C} \cdot 100\). On the basis of micropuncture studies in amphibians and mammals it is generally considered that all the filtered potassium is probably reabsorbed in the proximal part of the renal tubule. Therefore the potassium appearing in the urine may occur as a result of active secretion into the tubular lumen from the distal tubular cells. For this reason the excreted potassium was considered to have been secreted and was expressed as a percentage of the filtered load so that: percentage secretion of the filtered potassium = \(\frac{U_K v}{k P_K C} \cdot 100\), where \(U_K v\) was the excreted potassium (m-equiv./kg. body wt./day) and \(k P_K C\) was the filtered load of potassium (m-equiv./kg. body wt./day). The percentage reabsorption of water, osmoles, sodium and chloride and the percentage secretion of potassium by the renal tubules of the pre-smolting and post-smolting fish did not differ significantly from the corresponding values for smolting fish (Table 9). The plasma potassium concentrations of the pre-smolting and smolting trout were, however, extremely variable (Table 5). Consequently, the filtered loads of potassium, and in turn the percentage secretion of the filtered loads, were also very variable in these groups (cf. Tables 8 and 9).

**DISCUSSION**

During the period of smolting various species of salmonid fishes commence their anadromous migrations to the sea. Upon adaptation to the hypertonic marine environment these fish must modify their excretory processes to counteract the tendencies toward dehydration and excessive electrolyte intake. One aspect of this adaptation involves a drastic reduction in the urine flow rate (R. M. Holmes, 1961), which is apparently mediated through a lowering of the glomerular filtration rate (Holmes & McBean, 1963). The stimulus for the reduced urine flow and glomerular filtration rates has been generally considered to be associated with the rising environmental osmolality of the estuarine waters and the transient rise in plasma electrolyte concentrations observed after abrupt transfer of these fish to sea water. The present observations seem to suggest that a considerable reduction in both urine flow and glomerular filtration occurs during smolting. Certainly these fish were not exposed to increased electrolyte concentrations in the environmental fresh water. The work of Baggerman (1960), however, has indicated that increasing day-length is an important factor governing the appearance of the overt symptoms of smolting, and these findings have been confirmed by McInerney (1964).

The glomerular filtration rate of the non-smolting trout confirms an earlier value obtained by an indirect measurement of the rate of inulin clearance from trout maintained under identical conditions (Holmes & McBean, 1963). In these experiments a 25% reduction in the glomerular filtration rate was observed in fresh-water trout treated with corticosterone. The presently reported 48% reduction in the glomerular filtration rate of smolting trout raises the possibility therefore that adrenal steroids may influence the glomerular filtration rate at this time. In the smolting Atlantic salmon (*Salmo salar*) Fontaine & Hatey (1954) have observed increased circulating levels of 17-hydroxycorticosteroids and Olivereau (1962) has reported an increased adrenocortical volume. Furthermore, the total concentrations of the urine nitrogen and the plasma non-protein nitrogen were significantly higher in smolting than in
Excretion in trout

non-smolting trout, *S. gairdneri* (Holmes & Stewart, unpublished). These increases are consistent with the adrenocortical stimulation of gluconeogenic activity (Ingle, Prestrud & Nezamis, 1950). If the adrenocortical hormones are indeed responsible for the renal excretory adjustments occurring at smolting, then a stimulation of the pituitary–adrenal axis must also occur at this time. Increasing ACTH secretion in response to changes in photoperiod may well characterize the onset of smolting in salmonids. The attendant physiological adjustments, such as the modified renal excretory pattern, would then constitute a form of partial pre-adaptation to the marine environment, whereas changes in the *milieu intérieur* upon entry into sea water would be only secondary stimuli of the regulatory mechanisms responsible for the maintenance of homeostasis. Clearly, changes occur in the renal excretory pattern of the freshwater-adapted smolting trout which tend toward the pattern known to exist in the seawater-adapted individual.

In no case could the variations in urine flow between smolting and non-smolting trout, *S. gairdneri*, be attributed to selective changes in the water-reabsorptive processes in the nephron. The observed changes appeared to be entirely dependent upon variations in the glomerular filtration rate. Recent studies of the lamprey, *Lampetra fluviatilis*, have also indicated that variations in urine flow rate were directly related to changes in glomerular filtration and independent of tubular water reabsorption (Bentley & Follett, 1963).

The differences between mean urine electrolyte concentrations of the smolting and non-smolting trout were of a rather low order of significance in the present observations. In the smolting trout, however, where the urine flow rates were most variable, there was a significant negative correlation between the individual total osmolal concentrations of the urine and the individual urine osmolar excretory rates \((r = -0.74, P < 0.01\) with 24 degrees of freedom). On the other hand, there was a significant positive correlation between the total osmolal excretion and the total water excretion by these smolting trout \((r = 0.48, P < 0.01\) with 25 degrees of freedom). Thus it would appear that although there was a decrease in the osmolar concentration of the urine as the rate of urine flow increased, this decrease was insufficient to maintain the total osmolal excretory rate at a constant level. A somewhat similar relationship was observed by Bentley & Follett (1963) in the fresh-water lamprey, *L. fluviatilis*. These workers suggested that the decrease in the urine concentrations of sodium and potassium with increasing urine flow may have been due to increased tubular reabsorption. No such change, however, could be detected in the trout, *S. gairdneri*. Variations in the tubular secretion of nitrogenous compounds, however, may well have contributed towards this phenomenon.

The constant rate of urine flow by all groups of smolting and non-smolting trout strongly suggests that the freshwater-adapted trout is, in effect, being constantly water-loaded. If, therefore, the rate of urine flow reflected the osmotic influx of water from the environment, then the decrease in urine flow at the time of smolting may indicate a change in the permeability of the integument and/or the gill epithelium to water at this time.

Since the extracellular fluids of the fresh-water teleost are hypertonic with respect to the environment, a passive efflux of many ion species into the environment occurs (Krogh, 1939). Furthermore, the renal excretion of electrolytes by the fresh-water
teleost represents an additional drain on the stores of body electrolytes. To compensate for these renal and extra-renal losses there is a concomitant active uptake of ions from the environment. Since the trout used in this study did not have access to any dietary source of electrolytes for at least one week prior to the collection of urine, and since no net decrease of sodium and chloride concentrations in plasma and muscle was detectable by the analytical methods used, we must conclude that the renal excretion of these ions reflected their net uptake from the environment. If this line of reasoning is valid it would appear that sodium and chloride were being taken up at equimolar rates. Krogh (1937, 1939) first postulated that the active uptake of sodium and of chloride by the gill epithelium of fresh-water teleosts were independent mechanisms which were at least in part dependent upon the simultaneous exchange of ammonium and bicarbonate ions respectively. This hypothesis has recently been restated (Romeu & Maetz, 1964; Maetz & Romeu, 1964).

Although it does not confirm the hypothesis, the equimolar excretion of sodium and chloride in the urine is quite compatible with the independent uptake mechanisms responsible for the balance of this ion loss. With respect to potassium, however, Romeu & Maetz (1964) have reported a mean potassium efflux of 1.3 m-equiv./kg. body wt./day (range 0.2–4.4) from the gills of Carassius auratus maintained under a variety of experimental conditions. A net potassium efflux of this order of magnitude would quickly deplete the extracellular store of this ion. For instance, the plasma volume of the trout, S. gairdneri, has been reported as 130 ml./kg. body weight (Conte, Wagner & Harris, 1963) and therefore a 1 kg. saline-injected pre-smolting trout would contain approximately 0.022 m-equiv. plasma potassium. At the presently recorded rates of renal excretion this amount would be completely depleted in 3.8 hr., but an additional extra-renal efflux equivalent to the mean value reported for C. auratus would result in complete depletion after only 0.37 hr. A compensatory shift in the distribution of potassium from the intracellular to the extracellular space during a one-week starvation period would result in a depletion of intracellular potassium equivalent to a decline in muscle potassium concentration of approximately 10 m-equiv./kg. wet wt. Considering that the net extra-renal efflux rates of potassium recorded for C. auratus (Romeu & Maetz, 1964) were determined at an environmental temperature of 20°C, it is possible that the extra-renal efflux of potassium in S. gairdneri maintained at 4–10°C was considerably less. It may well be that the continuous renal excretion of potassium was accompanied by a similarly continuous extra-renal loss during the period of these experiments. The possibility therefore that a shift in the distribution of potassium within the body was responsible for the maintenance of the plasma potassium concentration is worthy of further study.

SUMMARY

1. A decline in the renal excretory rates of water and electrolytes occurred in the trout, Salmo gairdneri, at the time of smolting.
2. This decline appeared to be almost entirely attributable to a reduction in the glomerular filtration rate.
3. Although there was an increase in the total urine osmolal concentration as the urine flow decreased, this decrease was insufficient to maintain the total osmolal excretory rate at a constant level.
4. The sodium, potassium and chloride concentrations of the urine only accounted for approximately 50% of the observed real osmolality of the urine in both the smolting and the non-smolting fish; the difference may have been due to the presence of osmotically active nitrogenous compounds.

5. No selective change in the tubular reabsorptive or secretory processes were found to accompany the reduction in glomerular filtration at the time of smolting.

6. The urine:plasma-ion concentration ratios showed no evidence for any selective concentration in the renal tubule.

7. During the transition from the smolting to the post-smolting condition the renal excretory pattern was restored to that of the pre-smolting trout.

8. The possible significance of these changes in relation to the excretory changes known to occur during the adaptation of salmonid fishes to a marine environment are discussed.

REFERENCES


