Water and ion transport by the urinary bladder of the teleost Pseudopleuronectes americanus

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AN INCREASING WEIGHT of evidence suggests that the urinary bladder contributes significantly to osmoregulation in higher teleosts (12, 13, 17). However, the mechanisms for modifying urcteral urine and the possible beneficial effects of this modification have not been clearly demonstrated. Marine teleost urinary bladders have been shown to be capable of net mucosal- (lumen) to-serosal (blood) transport of Na and water as a hyperosmotic reabsorbate (15, 17). Active transport of both Na and Cl by the urinary bladder and show that this results in the concentration of divalent ions in the mucosal fluid. This process was inhibited by ouabain, furosemide, and ethacrynic acid. Evidence of acidification of the mucosal fluid and K secretion by the bladder indicate that it is a functionally more complicated structure than has been previously proposed.

MATERIAL AND METHODS

Animals. Winter flounder Pseudopleuronectes americanus, weighing about 200 g, were obtained by trawl in seawater just off the coast of Mount Desert Island, Maine. The animals were maintained at the laboratory in flowing seawater which had an osmotic pressure of 934 mosmol X kg\(^{-1}\) water and contained the following ions, in millimoles per liter: 343.0 Na; 8.8 K; 51.5 Mg; 9.8 Ca; and 486.1 Cl. Temperature ranged from 5 to 22°C in an annual cycle. Prior to use, animals obtained from seawater below 8°C were maintained at least 1 wk in a 10°C seawater bath (Frigid units).

Experimental technique. Two types of bladder preparations were used in these experiments. A sac-type preparation was used to measure water transport and associated net ion transport. A perfused bladder preparation was used for unidirectional ion flux measurements.

Unanesthetized animals were decapitated and the urinary bladder was dissected free of mesentry. For sac-type preparations, the ureteral end of the bladder was ligated. A flared PE-200 (Clay-Adams, Intramedic) tube was inserted near the ureteral end of the bladder and fastened by a rubber stopper (Fig. 1). This long glass tube was
necessary to prevent loss of fluid during the frequent vigorous contractions of the bladder. The stopper was equipped with a hook by which this entire assemblage could be weighed. The bladder was suspended in 100 ml of modified Forster’s saline (MFS) (10) plus 5.5 mM glucose (Table 1). The incubation medium was modified by omission of the biphosphate-bicarbonate buffer system and substitution of 3 mM imidazole (pH = 7.8). Possible leakage of the biphosphate-bicarbonate buffer system and Forster’s saline (MFS) (10) plus 5.5 mM glucose with a hook by which this entire assemblage could be used here to designate electrode bridges used to pass current through bladder wall and to monitor transepithelial electrical potential difference, respectively.

The ion transport inhibitors used were ouabain (Calbiochem, octahydrate, mol wt 728.8), furosemide (Lasix, injection, 10 mg/ml, Hoechst Pharmaceuticals, Inc.), and ethacrynic acid (free of mercurial preservatives, compliments of Merck Sharp & Dohme, assay = 99.63%). Each bladder served as its own control. In each case, inhibitor was added after two or three determinations of normal fluxes. In sac-type bladder preparations, the drug was added to both serosal and mucosal solutions. The effects of ouabain applied to the mucosal or serosal surfaces were examined in the perfused bladder preparation, but furosemide and ethacrynic acid were not tested further.

The transepithelial electrical PD was determined with double junction, Ag-AgCl reference electrodes (Orion Research, Inc., models 801 and 751). Voltage clamping electrodes consisted of Ag-AgCl wires communicating with the serosal and mucosal fluids via 3.0 M KCl-2% agar bridges, as shown in Fig. 1. The voltage supply for the voltage clamping electrodes was a Bioelectric NF-1 amplifier.

**Analytical methods.** In sac-type bladder preparations, a gravimetric technique was used to measure water loss or gain. The outer surface of each bladder was blotted thoroughly on filter paper prior to weighing to the nearest 0.05 mg (Mettler Instrument analytical balance model 16). To determine the amount of water loss or gain by the sac-type bladder preparation during an experimental period, the weights of the suspension apparatus plus the fluid-filled bladder were measured before and after each experimental period. Samples of the mucosal fluid were taken before the first weighing and after the second. The error of the weighing technique was tested by repeated rinsing, blotting, and weighing of a complete bladder assemblage. With uniform blotting and minimum exposure to air, the weight was reproducible to within 1 mg. Taking an average bladder weight of 50 mg and an average change in mucosal fluid volume of 25 μl, the weighing error would cause a percentage error of 4% in determining the water flux. For this reason, small bladders were usually incubated for 2 h per experimental period to increase the amount of change in mucosal fluid volume. At the end of an experiment, the bladder was cut from the suspension assemblage between the two fastening sutures, cut open, blotted gently on filter paper, and weighed. The remaining portions of the bladder and the suspension assemblage were also weighed. The initial and final volume of the mucosal fluid were determined by subtraction of the sus-
pensor weight plus bladder weight from the combined weight of suspensor, bladder, and mucosal fluid. The specific gravity of mucosal fluid was assumed to be unity for purposes of weight-to-volume conversion.

Na and K concentrations were determined by flame photometry with a precision of ± 1% (Instrumentation Laboratories, Inc. model 343). Cl concentration was determined coulometrically with a precision of ± 1% (Buehler-Cotlove Chloridometer). Osmotic pressure was determined by freezing-point depression with a precision of ± 1% (Precision Systems, Omsette A). Ca and Mg were determined by atomic absorption spectrophotometry (Perkin-Elmer model 107) after suitable dilution in 1% lanthanum oxide and 5% hydrochloric acid (precision ± 3%). Net H\textsuperscript{+} movement was determined from the change in pH and the concentration of buffer in the mucosal fluid. pH was determined on samples with a glass electrode (Arthur H. Thomas Co.) after brief agitation in air.

For measurement of unidirectional Na and Cl fluxes, 1 µCi of \textsuperscript{22}NaCl (New England Nuclear) or 4.75 µCi Na\textsuperscript{36}Cl (New England Nuclear) were added to 50 ml MFS. S-to-M flux was determined by adding isotope to the serosal medium and collecting the perfusion fluid and assaying total radioactivity. The specific activity of the serosal medium was measured before and after each flux measurement, and the change in specific activity was found to be negligible. M-to-S flux was determined by adding tracer to the perfusion fluid and subsequently determining the total radioactivity of the serosal bath. The serosal bath was renewed frequently to prevent backdiffusion of tracer. The tracer was found to have equilibrated with the tissue 15 min after initial exposure. Flux periods ranged from 15 to 30 min. \textsuperscript{22}Na was counted on a NaI(Th) well-type gamma scintillator (Nuclear Chicago Corp.). \textsuperscript{36}Cl was counted by liquid scintillation (Packard Tri-Garb).

The urinary bladder of the winter flounder is a tubelike expansion of the fused ureters which is highly contractile and distensible. For this reason, sac-type bladder preparations unavoidably had a continuously changing surface area due to continuously decreasing volume. Net fluxes in these preparations were, therefore, expressed in units of weight per centimeters squared. Surface area was determined from the specific activity of the mucosal fluid by the bladder.

Calculations. The net movement of each ion (\(\Delta Q_i\)) during the time \(t\) (h) across the sac-type bladder preparation was estimated as follows:

\[
\Delta Q_i = (C_i \times V)_{t_2} - (C_i \times V)_{t_1}
\]

where

- \(C_i\) = concentration of ion in mucosal fluid (µmol X ml\(^{-1}\))
- \(V\) = volume of mucosal fluid (ml)

Net flux (\(Q_i\)) (as µmol/g bladder wt per h) was then

\[
Q_i = \Delta Q_i \times g^{-1} \times h^{-1}
\]

Net H\textsuperscript{+} movement in sac-type bladder preparations was calculated by use of the Henderson-Hasselbalch equation, where pH and buffer concentration of the mucosal fluid are known. The buffer was 3 mM imidazole (weak base) which has a pKa of 6.92. The concentration of imidazole as the conjugate acid (a) in the mucosal fluid was equal to the total imidazole concentration (3 mM) minus the concentration of imidazole as the weak base (b), or:

\[
[a] = 3 \text{ mM} - [b]
\]

The [a] can be calculated by combining the Henderson-Hasselbalch equation:

\[
pH = pK_a + \log \frac{[b]}{[a]}
\]

with equation 3 and rearranging terms, so that:

\[
[a] = 3 \left(1 - \frac{K_a}{[H^+] | K_a}\right)
\]

The [a] was determined at the beginning and end of each experimental period. The change in [a] was due to the addition or removal of H\textsuperscript{+}, and the net flux of H\textsuperscript{+} was calculated with equations 1 and 2. Titrable acidity was not determined, and two assumptions were inherent in the calculation of the net flux of H\textsuperscript{+}. The first assumption was that imidazole was the only buffer present in solution, i.e., no buffering substance was produced and added to the mucosal fluid by the bladder in significant amounts. The second assumption was that imidazole was neither reabsorbed nor secreted by the bladder. Imidazole was not measured directly. Because of this assumption, the total concentration of imidazole in the mucosal fluid (3 mM) was corrected for the concentrating effect of fluid absorption by the bladder.

Unidirectional fluxes (\(J_{SM}\) or \(J_{MS}\)) of Na or Cl in perfused bladders were calculated from the specific activity (cpm/µmol) and the total radioactivity (cpm) as follows:

\[
J_{SM} = \frac{\text{total cpm}_{\text{M}}}{\text{cpm}_{\text{SM}}/\mu \text{mol}} \times \text{cm}^{-2} \times \text{h}^{-1}
\]

or

\[
J_{MS} = \frac{\text{total cpm}_{\text{S}}}{\text{cpm}_{\text{SM}}/\mu \text{mol}} \times \text{cm}^{-2} \times \text{h}^{-1}
\]

where the subscripts S and M refer to serosal fluid and mucosal fluid.

**RESULTS**

Transepithelial electrical potential difference of sac-type and perfused urinary bladders. Electrical potential difference measurements were necessary to establish active or passive ion transport. The time courses for the development of the equilibrium PD and the maximum PD developed by sac-type preparations were variable. Examples of the potential development are shown in Fig. 2. The same basic pattern was shown by all sac-type preparations with regard to the development of the PD. Immediately after introduction of the mucosal fluid, the PD was a few millivolts, mucosa positive. After a variable length of time, the mucosal side became more negative with respect to the serosal...
side. This pattern was repeated each time the mucosal fluid was renewed. The fact that NaCN (0.1 g/l) added to the external bath completely abolished the PD (Fig. 2) indicated that metabolic processes were necessary to maintain the PD. The PD measured across the sac-type preparation with initially identical solutions on the two sides averaged 23.4 ± 4.9 (SE) (n = 9) mV, mucosa negative (range: 10.4–33.1 mV) after an average of 4 h incubation. Continuous perfusion of isolated bladders maintained the PD at 4.4 ± 1.7 (SE) (n = 15) mV, mucosa positive (Table 3), and in these conditions the PD was stable (see example, Fig. 2). Thus, the negative PD resulting from sac-type preparations was probably attributable to the nature of passive ionic permeabilities and a concentration gradient set up by active transepithelial ion transport and water absorption. This negative PD cannot be maintained by active ion transport when the mucosal medium is continuously renewed by perfusion. Preliminary experiments have shown that in vivo the PD between urine and blood is about 4.0 mV, negative urine. Therefore, the sac-type preparation probably gives a better comparison of the in vivo function of the urinary bladder than the perfused bladder; however, to characterize the active or passive nature of Na and Cl transport, the perfused bladder was more useful because of the stable PD.

Characterization of unidirectional fluxes of Na and Cl in isolated perfused urinary bladders. Unidirectional fluxes of Na and Cl across perfused bladders in open-circuited conditions with identical solutions inside and outside showed that M-to-S flux was about 6 times the S-to-M flux for both ions (Table 2). Cl influx and efflux both averaged slightly higher than Na influx and efflux, but the net movements of the ions calculated as the difference in unidirectional fluxes averaged almost the same (Table 2).

The positive potential with respect to the mucosal surface made it possible to speculate that Cl was actively transported providing an electrochemical gradient down which Na moved passively. This prediction can be tested by elimination of the electrochemical gradient across the bladder wall. Under these conditions Na transport should be greatly reduced. The active transport potential (Eion) ought to be high for Cl and low or zero for Na. To calculate Eion for each of these ions, unidirectional fluxes were measured in short-circuited conditions with the same solution inside and outside. Thus, no electrochemical gradient existed across the bladder wall. Eion was calculated with the equation set forth by Ussing and Zerahn (23):

\[
E_{\text{ion}} = \frac{RT}{zF} \ln \frac{J_{\text{SM}}}{J_{\text{MS}}}
\]

where \( R \) = universal gas constant; \( T \) = absolute temperature (283°K); \( z \) = valence of the ion; \( F \) = Faraday's number; and \( J_{\text{MS}} \) and \( J_{\text{SM}} \) = unidirectional flux from mucosa to serosa and serosa to mucosa, respectively.

As can be seen in Table 3, the average values of \( E_{\text{Na}} \) and \( E_{\text{Cl}} \) are both between 40 and 50 mV, \( E_{\text{Na}} \) negative and \( E_{\text{Cl}} \) positive with respect to the lumen. As noted above, the measured PD of the perfused bladder averaged about 4 mV. This was fairly conclusive evidence that both Na and Cl are actively transported by the urinary bladder.

The relationship of Na transport to Cl transport is an important one in light of the small PD and substantial active transport of both ions. To examine this relationship more closely, unidirectional fluxes were measured while voltage clamping the bladder at transmucosal PD's of +50 and -50 mV. Table 4 shows that voltage clamping had no significant effect on either Na or Cl movement in either direction. Voltage clamping at 0 mV (short circuiting) had no effect on the transepithelial fluxes when compared to fluxes in the open-circuited state. A slight reduction in active flux (\( J_{\text{MS}} \)) was seen when the bladders were clamped at either -50 or +50 mV; however, the effect of the voltage clamp was the same on both Na and Cl transport.

Net water and associated net ion transport by sac-type bladder preparations. Perhaps the most important function of the urinary bladder is the absorption of water from the ureteral urine. Water transport could not be easily studied in the perfused preparation and, therefore, sac-type preparations were studied. The results verified that isolated sac-type bladder preparations, when incubated with initially identical solutions on the mucosal and serosal surfaces, were able to perform net fluid absorption (M to S) (Table 5). This net fluid transport was accompanied predominantly by net M-to-S transport of Na and Cl (Table 5). The rates of Na and Cl net transport were essentially the same, and the quantity of Na and Cl transported accounted for almost all of the net transport of osmotically
Table 3. Estimation of epithelial active transport potentials (E$_{ion}$) for Na$^+$ and Cl$^-$. in isolated, perfused urinary bladders of Pseudopleuronectes americanus.

<table>
<thead>
<tr>
<th></th>
<th>Na$^+$, nEq X cm$^{-2}$ X h$^{-1}$</th>
<th>Cl$^-$, nEq X cm$^{-2}$ X h$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.S.</td>
<td>J.M.</td>
<td>$E_{Na}$, mV</td>
</tr>
<tr>
<td>0.586</td>
<td>0.069</td>
<td>$-49.7$</td>
</tr>
<tr>
<td>1.397</td>
<td>8.343</td>
<td>$-43.6$</td>
</tr>
<tr>
<td>0.061</td>
<td>0.905</td>
<td>$-65.8$</td>
</tr>
<tr>
<td>0.690</td>
<td>5.005</td>
<td>$-39.7$</td>
</tr>
</tbody>
</table>

Mean ± SE 47.5 ± 6.2 (1) ⊕ 41.3 ± 4.7 (7)

Numbers in parentheses are numbers of observations. The unidirectional fluxes were obtained on short-circuited bladders with identical solutions on the two sides. Na$^+$ and Cl$^-$ fluxes were measured on separate bladders. The sign of $E_{ion}$ refers to the mucosal side. J.M. = serosal-to-mucosal flux. J.S. = mucosal-to-serosal flux. See text for equation used to calculate $E_{ion}$.

Table 4. Effect of voltage clamping on unidirectional fluxes of Na$^+$ and Cl$^-$ through isolated, perfused bladder of Pseudopleuronectes americanus.

<table>
<thead>
<tr>
<th>Imposed</th>
<th>Ion</th>
<th>J.M.</th>
<th>J.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD, mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>Na</td>
<td>1.086 ± 0.722 (5)</td>
<td>0.949 ± 0.190 (5)</td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td>0.955 ± 0.085 (7)</td>
<td>1.011 ± 0.128 (7)</td>
</tr>
<tr>
<td>+50.0</td>
<td>Na</td>
<td>1.009 ± 0.195 (4)</td>
<td>0.933 ± 0.158 (6)</td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td>1.101 ± 0.055 (7)</td>
<td>0.772 ± 0.056 (7)</td>
</tr>
<tr>
<td>-50.0</td>
<td>Na</td>
<td>1.140 ± 0.120 (4)</td>
<td>0.022 ± 0.093 (6)</td>
</tr>
<tr>
<td></td>
<td>Cl</td>
<td>0.966 ± 0.124 (6)</td>
<td>0.821 ± 0.122 (7)</td>
</tr>
</tbody>
</table>

All values are means ± SE. Numbers in parentheses are numbers of observations. The PD (transepithelial potential difference) was clamped at the indicated voltage (with sign referring to the mucosa) for 15-min periods during which the fluxes were measured. The fluxes are expressed as a ratio with the open-circuited (OC) flux determined before and after each clamping period. J.M. = flux from serosa to mucosa. J.S. = flux from mucosa to serosa.

Table 5. Net water and ion fluxes across wall of isolated sac-type bladder preparations of Pseudopleuronectes americanus.

<table>
<thead>
<tr>
<th></th>
<th>M to S</th>
<th>S to M</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>536.0 ± 122.9</td>
<td>5.13 ± 2.59</td>
</tr>
<tr>
<td>Osmotic substances</td>
<td>218.3 ± 47.1</td>
<td>1.91 ± 0.60</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>93.4 ± 20.1</td>
<td>0.54 ± 0.35</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>98.4 ± 23.3</td>
<td>(17)</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>0.06 ± 0.19</td>
<td>(17)</td>
</tr>
</tbody>
</table>

All values are means ± SE. Numbers in parentheses are numbers of observations. The rates of Na and Cl movement were not significantly different (Student t test). M and S refer to the mucosal and serosal surfaces, respectively.

The pH of the mucosal fluid was consistently decreased as a result of H$^+$ secretion (Table 5). However, it should be noted that the calculation of the quantity of H$^+$ secretion was made with the assumption that imidazole was not absorbed by the bladder. Therefore, the calculation was corrected for the increase in imidazole concentration that would result from fluid transport out of the bladder, but no direct measurement of imidazole was attempted.

The amounts of Ca$^{2+}$ and Mg$^{2+}$ moved in this type of experiment were of doubtful significance (Table 5). It should be noted, however, that the concentrations of these ions in the mucosal fluid almost always increased and more specific experiments presented below will illustrate the importance of this.

Relationship of urinary bladder transport processes to the possible in vivo role. To relate the ion transport processes of the urinary bladder to the possible in vivo function, the effect of presenting the mucosal surface with a Mg-rich solution was examined. Normally, the urine of marine teleosts contains a Mg concentration of from 50 to 200 mM and is slightly hypotonic to plasma (9). MgCl$_2$ was substituted for a portion of NaCl in the MFS so that the final osmotic pressure was slightly less than normal MFS (Table 1). The final Mg concentration of the mucosal fluid was 58 mM and the serosal fluid was 10 mM. Because of the importance of determining water movement under these conditions, the sac-type bladder preparation was used. Net transport, lumen to bath, of water, Na, and Cl still occurred (Table 6). Na and Cl were moved in equal amounts despite the fact that the Cl concentration of the mucosal fluid was almost 3 times higher than the Na concentration (Table 6). A net movement of K into the mucosal fluid occurred while small amounts of Mg and Ca moved from lumen to bath (Table 6). The Na concentration of the mucosal fluid decreased rapidly while the Cl concentration remained unchanged during these time periods. At these rates of movement, however, Cl should eventually become concentrated in the mucosal fluid. K concentration, of course, increased. Most significantly, the
concentrations of Mg and Ca were considerably increased in the mucosal fluid.

Effects of ion transport inhibitors on water and ion transport by isolated urinary bladders. The effect of $10^{-4}$ M furosemide on net water and ion transport by the urinary bladder with identical MFS bathing the two sides was not significant, although a tendency for reduction of fluxes was apparent (Fig. 4). To determine if the maximum effect of the drug had been achieved at the $10^{-4}$ M concentration, experiments were performed with $1.2 \times 10^{-3}$ M furosemide. At this concentration significant reductions in the net fluid and ion transport occurred (Fig. 4).

The effect of ethacrynic acid was similar to that of furosemide in that a concentration-dependent effect was apparent. At $10^{-4}$ M ethacrynic acid caused a reduction in net water, Na, and Cl transport but had a significant effect only on Na transport (Fig. 5). That the maximum effect of ethacrynic acid was not achieved at this concentration was shown by the fact that $10^{-2}$ M ethacrynic acid had a much greater effect (Fig. 5).

Comparison of the above drugs with the effect of ouabain showed that in this tissue they were comparatively weak inhibitors of ion transport. Ouabain ($10^{-4}$ M) very significantly reduced net transport of water, Na, and Cl, and osmotically active solutes from the lumen to the bath of sac-type bladder preparations (Fig. 6). The maximum inhibitory effect was evident within 1 h after incubation in ouabain solution. A comparable inhibitory effect was obtained by incubation of bladders with K-free MFS on the mucosal and serosal surfaces. The rate of net Na transport in controls and K-free bladders was 128.3 ± 36.6 (n = 12) and 15.1 ± 6.9 (n = 22) μeq X g⁻¹ X h⁻¹, respectively. In the same bladders net Cl transport was equally reduced from 126.1 ± 40.8 (n = 12) in controls to 17.5 ± 8.1 (n = 21) μeq X g⁻¹ X h⁻¹ in the bladders bathed in K-free MFS.

To further characterize the effect of ouabain on Na transport in the urinary bladder, unidirectional Na fluxes and PD were simultaneously monitored during exposure of the mucosal surface only to $10^{-4}$ M ouabain. The results are shown in Fig. 7. Ouabain applied only to the serosal surface simultaneously abolished the PD and the active portion of M-to-S Na flux (Fig. 7A). However, unexpectedly, exposure of only the mucosal surface to ouabain also inhibited the active portion of Na flux, yet had no effect on the PD (Fig. 7B, C, and D). Subsequently exposing the serosal surface of these same bladders to ouabain abolished the PD.
FIG. 4. Effect of $10^{-4}$ M and $1.2 \times 10^{-3}$ M furosemide on net water, Na, and Cl absorption (M to S) through wall of sac-type bladder preparations of *Pseudopleuronectes americanus*. An asterisk indicates significance at level of $P < 0.05$. Control values were obtained just prior to treatment with the drug. Numbers of determinations are shown in parentheses.

FIG. 5. Effect of $10^{-4}$ and $10^{-2}$ M ethacrynic acid on net water and ion movement through wall of sac-type bladder preparations of *Pseudopleuronectes americanus*. See Fig. 4 for explanation of graph.

**DISCUSSION**

Mechanism of Na and Cl transport. The electrophysiological and pharmacological data on the teleost urinary bladder reported here indicate that both Na and Cl are actively transported. The flux ratio ($J_{\text{Na}}/J_{\text{Cl}}$) for both Na and Cl was far from unity when the spontaneous PD was clamped at zero (short circuited) and identical solutions bathed the mucosal and serosal surfaces. These precautions do not eliminate possible entrainment of Na and Cl brought about by the active transport of some other substance; however, this is not likely since Na and Cl transport were very large compared to the net movement of any other ion. The only other factor which might have influenced transepithelial ion movement was the slight hydrostatic pressure maintained on the perfused bladder (see METHODS). The directionality of this force was M to S. That this pressure did not contribute to the M-to-S flux of Na and Cl was shown by the fact that $10^{-4}$ M ouabain equalized the unidirectional fluxes by abolishing the active portion of M-to-S flux (Fig. 7).

In addition to the fact that both Na and Cl appear to be actively transported, their movements through the epithelial membranes indicated that they are linked. Several factors point to this conclusion. The transepithelial PD of the perfused teleost urinary bladder averaged quite low in comparison to tissues such as toad bladder (24) or frog skin (23). In fact, on several occasions the active flux was quite high even though the PD was zero. It was expected that imposition of relatively strong electrical gradients across the bladder wall would impede the movement of one ion and aid movement of the other, depending on polarity. If both ions were actively transported from lumen to bath, the electrical gradients might not greatly influence flux in this direction. However, passive fluxes were expected to respond to the electrical gradients. The 15-min time periods were considered to be a sufficient period of time to determine whether or not the voltage clamps had any effect on flux because this was the maximum time ever necessary for isotopic fluxes to become stable after initial exposure of the tissue to $^{22}\text{Na}$ or $^{36}\text{Cl}$. Regardless of polarity, the imposed transmucosal PD's had almost no effect on active or passive Na or Cl transport.

What appears to be linked ion transport is not without precedent in other tissues. Na and Cl transport by the gallbladder appear to be inseparable and may be transported as a neutral complex (3). In the elasmobranch gastric mucosa H$^+$ and Cl$^-$ transport appear to be tightly linked (10). Neither of the above tissues generates a significant transepithelial PD; and, in the case of the gastric mucosa, the imposition of counter electrical gradients has no effect on the ion transport processes (10).

It would be premature to attempt to model Na and Cl transport by the teleost urinary bladder as has been done...
with other tissues (22) until other factors such as unidirectional K⁺ fluxes, intracellular ion concentrations, and the mechanism of H⁺ secretion have been determined. It may, however, be possible to state some of the components to be included in such a model. Na⁺ and Cl⁻ transport appear to be linked as in the gallbladder, but whether the coupling is nonelectrical (3) or electrical (13) cannot be definitely stated. Na-K-ATPase appears to be involved in Na⁺ (and Cl⁻) transport at both the mucosal and serosal surfaces of the epithelium; however, the ouabain-sensitive pump responsible, directly or indirectly, for the transepithelial PD is located on the serosal surface. Preliminary studies have shown that a direct correlation exists between the rate of active Na transport and the Na-K-ATPase activity of the teleost urinary bladder (19). This strongly implies the presence of an electrogenic Na⁺ pump and the electrical coupling of Na⁺ and Cl⁻ transport. The alternative would be active Cl transport coupled with active Na transport. The possibility exists that the mucosal ouabain effect was caused by penetration of ouabain to the serosal surface. In the particular experiments presented in this paper, the concentration of ouabain penetrating to the serosal surface would have to be high enough to specifically inhibit active Na transport but leave the ionic concentrations of the epithelial cells unaltered. Therefore, if the transepithelial PD were a diffusion potential, it would be unaltered. Tritiated ouabain binding studies and an autoradiographic study of tritiated ouabain binding in the teleost urinary bladder are now under way. These studies will help in the interpretation of the mucosal ouabain effect.

**Ion movements associated with water transport.** Prior studies on teleost urinary bladders did not indicate the composition of the absorbed fluid (M to S) with regard to divalent ions. The hypertonic fluid absorbed by the urinary bladder would be of no benefit to the intact animal if it were similar in composition to the mucosal fluid, i.e., if it contained equimolar concentrations of the primary urinary waste products, divalent ions. An important function of the kidney of marine teleosts seems to be the excretion of divalent ions (18). Ideally, this should be done with the minimum amount of water necessary because the fish is constantly losing water down an osmotic gradient.

**Fig. 6.** Effect of 10⁻⁴ M ouabain on net water, osmotically active solutes, and ion movements through sac-type bladder preparations of *Pseudopleuronectes americanus*. See Fig. 4 for explanation of graph.

**Fig. 7.** A: effect of exposure of only serosal surface of an isolated, perfused urinary bladder of *Pseudopleuronectes americanus* to 10⁻⁴ M ouabain. Transepithelial potential difference (PD), in millivolts, is shown by closed circles. Simultaneously measured mucosal- (M) to-serosal (S) Na⁺ flux (JNa), as μequiv cm⁻² X h⁻¹, is shown by bars. Width of bars indicates time interval during which flux was measured. Arrows indicate beginning of ouabain exposure. Dashed line in lower portion of each graph represents JNa. B, C, and D show effect of exposing only mucosal surface to ouabain with subsequent exposure of serosal surface.
to the ambient medium. The present study showed that net absorption of fluid by the urinary bladder was closely correlated with absorption of osmotically active solutes which were accounted for almost entirely by Na and Cl. The concentration of Mg in the final urine of marine teleosts is usually high and considerably variable (9), but even in the presence of a steep Mg gradient (M-to-S gradient was 58 to 1) the solute absorbed was nearly all Na and Cl, and as a result the divalent ions were concentrated in the mucosal fluid. In vivo, the Na and Cl of the absorbed fluid could be excreted by the gill, and the net result would be the retention of water.

The conservation of water by the bladder could be quite significant. For example, if the function and rate of ion transport of the bladder were the same in vivo as in vitro, a 200 g flounder would conserve about 3 ml x kg⁻¹ body wt x day⁻¹. If net diffusional water flux in P. americanus is similar to that measured in other fishes (5), the amount of water conserved by the bladder would amount to at least 10% of that flux. Despite the many assumptions, this example points out the possible importance of the bladder in overall salt and water balance of a marine teleost.

The physiological importance of the small K secretion by the bladder was not obvious. Most transporting epithelia studied lose some K through their mucosal surface (22). The K secretion found in the urinary bladder may be a passive leak; however, further experimentation will be necessary to establish its significance.

The amount of H⁺ secreted into the mucosal fluid by the teleost urinary bladder was greater than that seen in the toad bladder (7). In the toad bladder acidification of the mucosal fluid was somewhat dependent on carbonic anhydrase. This enzyme is also responsible for acidification in the turtle urinary bladder (21). The acidification of the mucosal fluid by the teleost urinary bladder appeared to be due to H⁺ secretion, but further examination of the acidification process will be necessary before the mechanism can be defined.

The ion movements described above are similar to those thought to take place in the distal tubules and/or collecting ducts of teleost kidneys. Lack of micropuncture data from teleosts prevents direct comparison of the tubular function of the kidney with the activities of the urinary bladder. However, the action of the urinary bladder of the fish on the mucosal fluid in vitro was superficially similar to the distal tubule function of the amphibian Amphiuma (25). In addition, the water permeability of the teleost urinary bladder was shown to be at least partially under the control of the hormone prolactin (12). Under the influence of this hormone, the water permeability of the bladder decreases and the Na concentration of the absorbed fluid increases. This is the opposite effect of vasopressin on the distal and collecting tubules of mammalian kidneys, but obviously serves the same purpose. It is, therefore, not unlikely that prolactin also influences the water permeability of the distal tubules and collecting ducts of teleosts.

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The present study showed that both furosemide and ethacrynic acid were effective inhibitors of ion transport by the teleost urinary bladder. However, ouabain was effective at much lower concentrations. As mentioned above, the rate of active transport of Na by the teleost urinary bladder was strongly correlated with Na-K-ATPase activity. This explains the effect of ouabain on ion transport, but it is unclear if the other drugs are simply less potent inhibitors of Na-K-ATPase or if they inhibit in some other way. The effect of furosemide and ethacrynic acid may be far greater in certain segments of the kidney tubules than it was in the urinary bladder.

The effects of inhibitors on the urinary bladder shown here, therefore, allow some prediction as to the effects of these substances in vivo on urine formation. Ouabain should cause diuresis in teleosts at least by its action on the urinary bladder and probably by action on distal and collecting tubules. If ethacrynic acid and furosemide cause diuresis in teleosts, it will probably result from their action on proximal or distal segments.

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