Sodium and chloride transport in tadpoles of the bullfrog *Rana catesbeiana*

RONALD H. ALVARADO AND ANNE MOODY

Department of Zoology, Oregon State University, Corvallis, Oregon 97331

Sodium and chloride transport in tadpoles of the bullfrog *Rana catesbeiana*. Am. J. Physiol. 218(5): 1510–1516. 1970.—Unfed bullfrog tadpoles actively transport sodium and chloride ions into their body fluids from a dilute bath. The rate of exchange is about 1 μmole/10 g-hr for each ion. This can be enhanced by salt depletion. Sodium and chloride can be transported independently each in exchange for an endogenous ion of like charge. Before stage XXII the body fluids are negative to the bath (1 mM NaCl) by 5–10 mV. At stage XXII and thereafter, the body fluids are positive. This correlates with the appearance of a potential difference and the active transport of sodium across the isolated skin. The electrical resistance of the skin also increased markedly at stage XXII. Tadpoles drink the medium at a rate of 0.14 ml/10 g-hr and absorb about half of the fluid ingested. However, the gut probably does not play a major role in sodium and chloride balance. Sodium and chloride are actively transported from fluid perfused through the gill chambers. This transport is enhanced by salt depletion.

The above data indicate the absence of active salt absorption in tadpoles. However, evidence collected in this laboratory shows that this conclusion is untenable (2). The objective of this report is to present evidence that transport of both Na\(^+\) and Cl\(^-\) into tadpoles living in dilute salt solutions is thermodynamically active.

MATERIALS AND METHODS

Animals. Bullfrog tadpoles, at various stages of development, were collected near Corvallis, Oregon. They were staged as described by Taylor and Kollros (27) and kept, unfed, in dechlorinated tap water (containing 0.2–0.4 mM NaCl) or in artificial pond water (1.3 mM NaCl, 0.8 mM CaCl\(_2\), 0.1 mM KCl, and 0.2 mM NaHCO\(_3\)). The temperature was 20–24°C but was controlled within ±0.5°C for a given experiment.

Analyses. Serum was diluted 100 times and analyzed for Na\(^+\) and K\(^+\) by flame photometry (precision ±1%) and for Cl\(^-\) with an Amino-Gotoflo chloridometer (precision ±1%). Total solute was determined on undiluted samples of serum with a Mechrolab vapor-pressure osmometer (precision ±3%). The concentration of ammonia in the bath was determined by direct nesslerization of 2-ml samples (precision ±2%). Ammonia determinations were made only over the first 6–12 hr after placing the animals in a fresh (ammonia-free) bath.

Fluxes. Sodium influx was measured with \(^{22}\)Na. The isotope (1 μC) was added to a bath of 100 ml, and 1.0-ml samples were withdrawn after specified intervals. Each sample was transferred to a planchet, evaporated to dryness, and the time required to accumulate 10,000 counts was determined with a gas-flow Geiger counter (precision ±1%). Separate bath samples were analyzed for [Na\(^+\)] by flame photometry and the net flux (M\(_{net}\)) was determined from these values. The influx (M\(_i\)) was calculated from the relation:

\[
M_{net} = M_i - M_o
\]

Chloride 36 was used to measure Cl\(^-\) fluxes. The isotope, obtained as HCl, was neutralized and 1 μC was added to the...
bath. Fluxes were measured as described for Na\(^+\) except that [Cl\(^-\)] was determined by electrometric titration.

**Potential.** The potential difference between the inside of the animal (coelomic fluid) and the bath was measured as described by Dietz et al. (8). Appropriate corrections were made for electrode asymmetry. The animals were anesthetized to 0.1% tricaine methanesulfonate and then transferred to a solution containing 1% urethan and 5 mM K\(_2\)SO\(_4\) as a conducting medium. Urethan does not affect the potential of adult anurans (13).

**Isolated skin.** The potential difference (E) and short-circuit current (I) across isolated skins bathed on each side with frog Ringer solution (110 mM NaCl, 1.9 mM KCl, 1.4 mM CaCl\(_2\), and 2.4 mM NaHCO\(_3\)) were measured as described by Ussing and Zerahn (29). Resistance across the skin was obtained by plotting E vs. I, as described by Myers et al. (20). Potentials were read with a Radiometer pH 4 potentiometer (±1 mV). Influx and efflux of Na\(^+\) and Cl\(^-\) were measured with \(^{22}\)Na and \(^{36}\)Cl, respectively. The isotope (1 \(\mu\)C) was added to the solution bathing one side of the skin (side A) and samples were drawn each hour from the affluent solution bathing the opposite side (B). The flux was determined by dividing the rate of appearance of radioactivity in solution B by the specific activity of solution A. Influx and efflux were measured on separate skins.

**Salt depletion.** In many aquatic animals exposure to distilled water (salt depletion) activates the ion pumps (16). We salt depleted tadpoles by keeping them in flowing distilled water (0.5 liter/animal per day). Animals survive several weeks in this medium.

**Drinking.** Tadpoles were placed in a bath of tap water with inulin-\(^{14}\)C added (1 \(\mu\)C/100 ml). After specified intervals animals were removed, rinsed, anesthetized, and the digestive tract was removed. Wet and dry weights of the digestive tract (with contents) were obtained. The dried tissue was pulverized and placed in a flask. Water was added, and the inulin was extracted at 2 C for 24 hr. After centrifugation, a sample of the supernatant and the bath was plated for radioactivity (10,000 counts). The rate of drinking was estimated from the relation:

\[
\text{drinking rate} = \frac{\text{total counts per minute in gut}}{\text{counts per minute per milliliter in bath} \times \text{time(hr)}}
\]

The rates were standardized to 10 g of animal. Serum was also checked for radioactivity; none was found.

**Perfusion of gill chamber.** Tadpoles were tranquilized in 0.1% tricaine methanesulfonate. A polyethylene tube (PE 240) was tied into the mouth so that fluid could be perfused into the mouth, through the gill chamber, and out the spiracle. The animals were placed, ventral side up, on a piece of rubber screening so that fluid leaving the gill chamber dropped into a container below with a minimum of contact with the exposed skin. The ventral part of the animal was covered with a moist piece of cotton and the whole container was covered. The perfusion fluid contained tap water or pond water plus 0.5% urethan and the radioactive ion of choice. By comparing the radioactivity of the affluent and the effluent solutions and measuring the perfusion rate, it was possible to estimate the influx of the ion in question:

\[
M_i = \frac{F(R_a - R_e)}{R_a/C_i}
\]

where: \(M_i = \) influx, \(\mu\)Eq/hr; \(F = \) rate of perfusion, ml/hr; \(R_a, R_e = \) radioactivity of affluent and effluent solutions, respectively, counts/min per milliliter; \(C_i = \) concentration of ion in affluent solution, \(\mu\)Eq/ml.

The difference between \(R_a\) and \(R_e\), which is the critical measurement in these experiments, depends upon the flow rate (F). After preliminary experiments, we selected a flow rate of 25 ml/hr. This provided a measurable difference between \(R_a\) and \(R_e\) at least for Na\(^+\) and at the same time kept the animals alive throughout the experiments (3–4 hr). We have not been able to find references on the normal rate of ventilation of the gill chambers of tadpoles. Samples of the affluent solution were taken at 10- to 20-min intervals. After each sample, the lower container (effluent) was emptied. The affluent solution was sampled at the beginning and the end of each experiment. Duplicate samples of each solution were plated on aluminum planchets and counted (10,000 counts). The precision of plating and counting was ±1%. The analytical error associated with the measurement of \(M_i\) over a single interval may be as high as ±40%. Chemical analyses for [Na\(^+\)] and [Cl\(^-\)] in the affluent and effluent solution provided an estimate of \(M_{\text{net}}\). The efflux was calculated from equation 1.

As a check we perfused the gill chambers with solutions containing inulin-\(^{14}\)C or \(^{35}\)S-labeled Na\(_2\)SO\(_4\), neither of which was expected to penetrate epithelia and found that \(R_a\) and \(R_e\) did not differ by more than 2% (n = 8). The average deviation from the mean of 10 samples of effluent solution (\(R_e\)) measured over 3 hr was less than 1% of the mean. When \(^{22}\)Na was tested \(R_e\) was 5–8% lower than \(R_a\) (F = 25 ml/hr).

**RESULTS**

**Ionic composition of body fluids.** Figure 1 shows the [Na\(^+\)], [Cl\(^-\)], and total solute concentration in serum of bullfrogs.
at various stages. There is a curious reduction in all three parameters between stages XIV and XVII for which we have no explanation. Potassium concentration in serum is $4.3 \pm 0.02$ mEq/liter ($n = 74$) with no observable difference with respect to stage. Sodium, Cl$^-$, and K$^+$ account for 85–90% of the total solute in serum.

Relative to tap water or pond water (PW), serum is markedly hyperosmotic and hyperionic with respect to Na$^+$ and Cl$^-$. The ratio of concentration in serum to concentration in bath ($C_{i}/C_{o}$) for animals in tap water is over 400 for both Na$^+$ and Cl$^-$. In PW it is 70 for Cl$^-$ and 80 for Na$^+$. Since these ratios are maintained in the absence of food and these ions are exchanged with the bath (see below) one of them must be actively transported and a sizable transepithelial potential difference would have to exist in order for either of them to be passively distributed.

**Potential difference—in vivo.** In adult bullfrogs, stage XXV, the body fluids are positive to the outside solution. The magnitude of the potential difference is a function of [NaCl] outside as shown in Fig. 2. At 1.5 mM Na$^+$ (PW) the potential difference is 15 mV (inside positive). This positive potential appears abruptly at about stage XXII. Earlier than this, the inside is negative to dilute bathing solutions (–5 to –10 in PW). As Na$^+$ is increased in the bath, there is a slight decrease in negativity in tadpoles, but the inside does not become positive over the concentration range we studied. For tadpoles in Na$_2$SO$_4$ solutions, the pattern is similar to that in NaCl. At 0.5 mM Na$_2$SO$_4$ the body fluids were negative to the bath by 5–12 mV ($n = 3$). In 1 mM KCl solution the body fluids were also negative by 5–10 mV ($n = 3$).

**Ion exchanges.** Figure 3 shows the net exchanges of Na$^+$, Cl$^-$, and K$^+$ between a representative tadpole and its bath (tap water) over a period of 3 weeks. There is an initial net loss of Na$^+$ and Cl$^-$ probably associated with handling, followed by a period during which a steady state is approximated for Cl$^-$ and the net loss of Na$^+$ is very slow (about 0.1 μEq/hr). Potassium is lost at a rate of about 0.3 μEq/hr with no apparent tendency to approach a steady state.

Figure 4A shows the influx and efflux of Na$^+$ in tadpoles acclimated to PW. There is a slight negative Na$^+$ balance ($M_{\text{in}}/M_{\text{out}} = 1.2$). But the magnitude of $M_{\text{in}}$ is small relative to $M_{\text{out}}$ and $M_{\text{out}}$. Animals salt depleted for 1 week and then placed in PW show a positive Na$^+$ balance. The time course is shown in Fig. 4B. Salt depletion primarily affects $M_{\text{out}}$, although after 12 hr there is a reduction in $M_{\text{out}}$, possibly reflecting a reduction in the renal loss of Na$^+$. The influx into salt-depleted tadpoles declines exponentially, reaching control values after about 2 days.

Tadpoles transferred from PW to 0.5 mM Na$_2$SO$_4$ display...
a negative Na⁺ balance (M₀/Mᵢ = 1.45), but there is a
tendency toward a steady state after 2 days (Fig. 4C). The
influx is comparable to that of animals in PW. Salt-depleted
tadpoles experience a net uptake of Na⁺ from 0.5 mM
Na₂SO₄, which can be attributed primarily to an elevation in
Mᵢ (Fig. 4D).

In no case does M₀/Mᵢ exceed 2 in the above experiments
whereas C₁/C₀ exceeds 65 in all cases. In order for the flux-
ratio equation to be satisfied with respect to Na⁺, the body
fluids would have to be negative to the bath by 87 mv (28).
This is at least 5 times greater than the potential measured
in intact tadpoles under our experimental conditions, indicat-
ing that Na⁺ is actively transported.

Sodium transport can be independent of Cl⁻ transport. In
separate studies we found that SO₄²⁻ (35S-labeled) penetrates
very slowly into intact tadpoles. Thus the inward transport
of Na⁺ probably involves exchange with an endogenous
cation, possibly NH₄⁺ or H⁺. Ammonia excretion in tadpoles
(stages X-XV) in 1 mM NaCl is about 3.5 ± 0.5 pmoles/
10 g-hr (n = 10).

Figure 5, A and B, shows the Cl⁻ fluxes in PW-acclimated
and salt-depleted tadpoles immersed in PW. The PW-ac-
climated animals approach a steady state whereas the salt-
depleted animals enjoy a positive Cl⁻ balance. There is also
a Cl⁻ influx from 1.0 mM KCl comparable in magnitude to
that in PW, and salt depletion stimulates the uptake (Mᵢ)
of Cl⁻ from KCl (Fig. 5, C and D). Application of the flux-
ratio equation reveals that transport of Cl⁻ from a bath of
1.0 mM NaCl or 1.0 mM KCl to the body fluids of tadpoles is
an active process (28). Potassium is lost during the net up-
take of Cl⁻ from 1.0 mM KCl so that, under these conditions,
Cl⁻ transport involves exchange with another anion.

Table 1 shows the flux values for recently transformed
bullfrogs (stage XXY). Sodium and Cl⁻ fluxes are compa-
rable to those of tadpoles and the flux-ratio equation is not
satisfied for either Na⁺ or Cl⁻, confirming the observations
of Jørgensen et al. (13) that frogs actively transport Na⁺
and Cl⁻.

<table>
<thead>
<tr>
<th>Ion</th>
<th>n</th>
<th>Weight, g</th>
<th>Flux, d³q/10 g-hr</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>M₀</td>
</tr>
<tr>
<td>Na⁺</td>
<td>3</td>
<td>7.7 ± 2.1</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>4</td>
<td>8.1 ± 0.7</td>
<td>2.4 ± 2.0</td>
</tr>
<tr>
<td>K⁺</td>
<td>7</td>
<td>7.9 ± 1.4</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>7</td>
<td>7.9 ± 1.4</td>
<td>1.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values represent means ± SE.

Table 2. Electrical properties and ion fluxes
across isolated skin

<table>
<thead>
<tr>
<th>Stages</th>
<th>Potential Difference, mv</th>
<th>Resistance, ohm-cm²</th>
<th>Flux, dEq/cm²</th>
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<tr>
<td></td>
<td></td>
<td>M₀</td>
<td>Mᵢ</td>
</tr>
<tr>
<td>VII-XVI</td>
<td>0</td>
<td>760</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>±51</td>
<td>±0.28</td>
<td>±0.33</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(9)</td>
<td>(8)</td>
</tr>
<tr>
<td>XVII-XXI</td>
<td>0</td>
<td>500</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>±60</td>
<td>±0.13</td>
<td>±0.15</td>
</tr>
<tr>
<td></td>
<td>(32)</td>
<td>(21)</td>
<td>(14)</td>
</tr>
<tr>
<td>XXII-XXIII</td>
<td>14</td>
<td>1,298</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>±2</td>
<td>±0.23</td>
<td>±0.05</td>
</tr>
<tr>
<td></td>
<td>(15)</td>
<td>(12)</td>
<td>(5)</td>
</tr>
<tr>
<td>XXV</td>
<td>25</td>
<td>1,710</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>±4</td>
<td>±0.10</td>
<td>±0.05</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(12)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Numbers in parentheses are
number of observations.

These experiments show that tadpoles share with adults
the capacity to actively transport Na⁺ and Cl⁻. The ques-
tion of the site of transport then arises. The skin, gills, and
gastrointestinal tract seem likely possibilities.

Isolated skin. We confirmed Taylor and Barker’s (26) ob-
servation that the potential difference across isolated skin
is absent in tadpoles and appears during metamorphosis at
about stage XXII. We have also measured unidirectional fluxes
of Na⁺ and Cl⁻ across the skin (Table 2). Variability was
high, particularly in young animals, but it is clear that be-
tween stages XVII and XXI Mi and MO of Na⁺ are not sig-
ificant different, (Mi/M₀ = 1.2). Whereas, above stage
XXI, Na⁺ influx exceeds efflux (Mi/M₀ = 4.3) despite the
fact that the potential difference is about 15 mv (inside
positive). The elevated ratio reflects primarily a drop in MO.
The Cl⁻ data are more variable. Between stages XVII and
XXI, Mi/M₀ = 1.2, and between stages XXII and XXV,
Mi/M₀ = 1.1. Under these experimental conditions (Ringer
solution on both sides), Cl⁻ is not actively transported
across the skin even in the adult stage.

Correlated with the appearance of the Na⁺-transport
system is a marked increase in the resistance of the skin
(Table 2). This is also reflected in the Na⁺ and Cl⁻ fluxes
which are reduced. Apparently one of the features associ-
of tadpoles of Rana catesbeiana was 0.14 ± 0.02 ml/10 g-hr (n = 7). Measurements were depleted 1 week.

The epithelium is a decrease in its permeability to ions. Measurements were clearly present. The potential difference between the body and the bath does not approach that required by the development of the potential across the isolated skin as an ion in affluent solution; ND = not depleted; SD = salt depleted 1 week.

Living in pond water without food, larval bullfrogs are living in pond water without food, larval bullfrogs are

Potassium is lost at a slow rate and, in nature, must be replaced by feeding. Sodium and chloride balance can be maintained in the face of concentration gradients exceeding 100, whereas the flux ratio (MNa/MCl) for these ions seldom exceeds 2. The flux ratio equation is not satisfied for either ion so that both must be actively transported. Sodium and chloride exchange at a rate of about 24 μmoles/10 g-day (PW-acclimated tadpoles in PW) which corresponds to about 5% of the total Na+ in a 10-g animal and about 14% of the total Cl-. The rates can be increased at least 3 times by pretreating the animals in distilled water. This salt-depletion response is characteristic of a number of freshwater animals (16). The mechanism is not clearly understood but endocrine control may be involved (5).

The active transport of Na+ and Cl- into tadpoles may occur independently. Since Na+ can be accumulated from Na2SO4 solutions and SO42- is not taken up at a measurable rate (unpublished data) there must be an exchange mechanism. A Na+-NH4+ exchange system has been proposed for crayfish (23), teleost fish (18), and adult frogs (16). Tadpoles excrete ammonia at a rate commensurate with such a system. On the other hand adult frogs are ureotelic, excreting about 15% of their total nitrogenous waste as ammonia (19). The existence of a Na+-NH4+ exchange system in adult frogs has recently been questioned because of the low ratio between NH4+ excreted and Na+ absorbed through the skin. At least in some species there is evidence for a Na+-H+ exchange system (10, 11). We found that adult bullfrogs (stage XXV) excrete ammonia at a rate of about 0.5-1.0 μmole/10 g-hr when they are in PW or 0.5 mM Na2SO4. This is a maximum rate because of the possibility of decomposition of other nitrogenous wastes over the 5-10 hr of measurement. Nevertheless it seems possible that at least a fraction of the Na+ exchanged could be with endogenous NH4+.

Net quantities of K+ are lost from tadpoles during the net accumulation of Cl- from 1.0 mM KCl. This indicates an anion exchange system. We have not analyzed this but it is possible that a Cl--HCO3- or a Cl--OH- exchange could occur as has been suggested for some other freshwater animals (11, 13, 18, 21, 24).

A correlate of active ion transport in most freshwater animals which have been studied is the development of an electrical potential difference between the body fluids and the bath. The inside may be negative as in the eel in freshwater (17), or positive as in adult anurans and larval and adult urodeles (3, 8, 13). In larval anurans (in freshwater) the body fluids are negative to the bath by 5-10 mv. The ionic basis for this potential has not been determined.

During late metamorphosis (stage XXVI) the inside becomes positive to the bath, and there is a large body of experimental evidence that this potential is generated by active transport of ions across the skin (29). The appearance of the potential in vivo at stage XXVI correlates perfectly with the development of the potential across the isolated skin as described by Taylor and Barker (26) and confirmed by us. Of course, a low potential difference or the absence of a potential does not preclude the possibility of active ion transport. Diamond (7) has shown that transport of Na+ and Cl- across the mammalian gall bladder is active but that there is no potential difference across the membrane.

Flux studies on isolated skins bathed on both sides with

<table>
<thead>
<tr>
<th>Ion</th>
<th>Treatment</th>
<th>n</th>
<th>Cx, mM</th>
<th>Flux, μEq/10 g-hr</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>Na</td>
<td>ND</td>
<td>6</td>
<td>0.2</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2</td>
<td>0.2</td>
<td>1.5 and 1.6</td>
</tr>
<tr>
<td>Cl</td>
<td>ND</td>
<td>4</td>
<td>0.6 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>5</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.6</td>
</tr>
</tbody>
</table>

Values represent means ± se. Animals were between stages XI and XVI. The rate of perfusion was 25 ml/hr. Cx = concentration of ion in affluent solution; ND = not depleted; SD = salt depleted 1 week.

Discussion

Living in pond water without food, larval bullfrogs are able to maintain Na+ and Cl- balance for prolonged periods.
Salt depletion enhances transport in the gill chamber and Na⁺ transport. This contention is supported by the facts that chamber show that this may be an important site of active are not major sites of ion transport between the external and the bath and the animal, the gills remain as the only exposed area with a large surface area. Studies on the perfused gill medium at a relatively high rate. This would appear to compound their osmoregulation problem since at least half of the water ingested is absorbed. The ingestion rate is too slow and the bath too dilute for the gut to be a major site of ion uptake in the unfed animal. Of course, in nature food is ingested with water which probably increases the availability of ions.

Since the skin and the gastrointestinal tract apparently are not major sites of ion transport between the external bath and the animal, the gills remain as the only exposed area with a large surface area. Studies on the perfused gill chamber show that this may be an important site of active Na⁺ transport. This contention is supported by the facts that salt depletion enhances transport in the gill chamber and that the injection of cardiac glycosides inhibits transport. These compounds are known to inhibit active Na⁺ transport in a variety of tissues (4, 22, 25). The rate of uptake of Cl⁻ in the gill chamber is variable. Of course, in nature food is ingested with water which probably increases the availability of ions.

Unlike most freshwater animals, tadpoles ingest the medium at a relatively high rate. This would appear to compound their osmoregulation problem since at least half of the water ingested is absorbed. The ingestion rate is too slow and the bath too dilute for the gut to be a major site of ion uptake in the unfed animal. Of course, in nature food is ingested with water which probably increases the availability of ions.

The differences in ionoregulation mechanisms in larval and adult bullfrogs raise some interesting questions. For example, what are the morphological and biochemical changes in the skin associated with the appearance of the ion-transporting systems in the skin? Second, what is the nature of the transport systems in the gills, particularly with reference to the capacity to transport Na⁺ and Cl⁻ independently? These problems are being investigated.

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