Renal function during osmotic stress in the aquatic toad *Xenopus laevis*

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McBean, Ralph L., and Leon Goldstein. Renal function during osmotic stress in the aquatic toad *Xenopus laevis*. Am. J. Physiol. 219(4): 1115-1123. 1970.—*Xenopus* toads, which are normally aquatic and amnonetelc, became amnoelc after maintenance in a hyperosmotic saline solution for 2-3 weeks. In addition, blood urea levels were elevated more than 15-fold, and this increase contributed significantly to achieving osmotic balance in the animals. Within a few hours after transfer of the toads to saline, urine flow fell to 1/10 of the rate in freshwater. By 7 days after transfer, flow had returned to about one fourth of the freshwater level, where it remained. Changes in glomerular filtration rate paralleled those in urine flow. It was estimated that the reduction in urine flow was essential to the observed urea accumulation; however, an increase in net urea synthesis also appeared necessary. The suppression of urine flow in *Xenopus* after transfer to saline appeared to be related to an increase in plasma sodium concentration. Dehydration per se did not occur.

urea, ammonia, sodium; glomerular filtration

THE SOUTH AFRICAN TOAD, *Xenopus laevis*, normally inhabits freshwater. In this habitat, it excretes about 3/4 of its waste nitrogen as ammonia (3). Under conditions of water deprivation (such as occur in natural estivation and when the toad is kept in hyperosmotic saline solution or on moist peat), there is an accumulation of urea in the tissues of the animal often to a level of 15 times normal, while the concentration of ammonia increases but slightly (4, 5, 15, 22). Thus, *Xenopus* shares with certain other amphibians and with members of other vertebrate groups the property of accumulating urea in response to water deprivation (10, 12, 13, 24, 33).

Two physiological processes may act, alone or in synergy, to raise the concentration of urea in the tissues of an animal. These are a) an increase in the rate of synthesis of urea, and b) a decrease in the rate of its excretion. Some evidence has been found for the occurrence of increased urea synthesis by *Xenopus* during water deprivation (4, 15, 16, 22). But until now there has been little exploration of the extent to which urea accumulation could be the consequence of a reduction in the urea clearance. Certainly, excretory factors appear to be involved in determining levels of urea in some other vertebrates which accumulate it. For example, recent studies by Goldstein, Oppelt, and Maren (10) indicate that, in the lemon shark (*Negaprion brevirostris*), the precise regulation of urea concentration is almost entirely governed by the kidney and/or the gills.

In *Xenopus*, there appears to be little active renal transport of urea, nor is there much extrarenal loss of this compound (3). The rate at which urea is cleared by this toad should therefore be related to GFR and urine flow. The amphibian kidney responds dramatically to dehydration and osmotic stress by reducing urine flow. For instance, when the bullfrog (*Rana cestans*) is removed from water, there is a prompt decline in urine production, a result both of reduced filtration and of enhanced fractional reabsorption of water (28); also the urea clearance is markedly reduced. Urine flow also declines when frogs are placed in hypertonic solutions of sodium chloride (1). *Rana cestans*, the “crab-eating frog” of southeast Asia, is the only salinotolerant frog in which the response of renal function to a change in ambient osmolarity has been studied. In this species, plasma urea concentration rises when the external salinity is increased, and this urea accumulation appears to have an osmoregulatory role. Schmidt Nielsen and Lee (29) found that urine flow in *R. cestans* diminished sharply as environmental salinity was raised, and they calculated that the decrease was due mostly to an increased tubular reabsorption of water, but also partly to a decreased GFR. These authors considered the suppression of urine flow to be the primary cause of the urea accumulation in elevated salinities.

The object of the present study was to evaluate the contribution of the kidney to osmoregulatory urea accumulation by *Xenopus*. First, the time course of adaptation was determined by following the concentrations of urea, ammonia and sodium in the blood for a 3-week period after transfer to hyperosmotic saline. Then, changes in nitrogen excretion, urine flow, and glomerular filtration rate were studied over the same time period. The concentration of sodium in the plasma, as well as some indices of the state of hydration (hematocrit, plasma protein, and inulin space) were also monitored.

METHODS

Animals

South African toads (male *Xenopus laevis* Daudin, weighing about 30 g) were purchased from a commercial importer (Jay E. Cook, Cockeysville, Md.). The animals were routinely kept in large glass aquaria containing recirculated filtered tap water at 23-27°C. Photoperiod was a constant 12 hr on, 12 hr off, whenever possible. The toads were fed
whole live earthworms once a week. They were fasted for at
least 10 days prior to the start of any experiment and for 18
days prior to experiments involving measurement of blood
concentration or excretion rate of ammonia or urea.

During experimental periods, each animal was kept in a
glass or polyethylene jar containing about .5 liter of aged tap
water or a saline solution. Water temperature was held be-
tween 24 and 26 °C. The medium was replaced daily.

Osmotic Stress

Hyperosmotic saline solution was selected as the agent for
water deprivation because long-term reproducibility seemed
simpler to achieve than with desiccation in peat. The saline
solution had an osmolality of approximately 300 mOsm/liter
and the following ionic composition: Na+, 150 mEq/liter; K+,
3.4 mEq/liter; Ca++, 6.8 mEq/liter; Cl-, 160 mEq/ liter;
and HCO₃⁻, 0.6 mEq/liter. This saline solution is
hyperosmotic to normal (freshwater) Xenopus plasma (233
mOsm/liter, Table 2).

Sampling for Analysis

Excretion. Excretory products were sampled in two ways.
The first was simply to remove and analyze serial samples of
the medium into which the animal was urinating. A toad
was placed in a glass jar containing 500 ml of water or saline
buffered at pH 6.5 with sodium phosphate (0.01 m); oxy-
tetracycline HCl (Terramycin: 0.5 ml of 0.02%/500 ml,
Pfizer) was added to retard bacterial decomposition when
ammonia and urea were to be measured (3).

The second way of sampling excretory products was to
obtain urine through a catheter. Toads were lightly anes-
thetized with tricaine methane sulfonate (MS 222, Sandoz),
0.5% in the appropriate medium. A flared end of a piece of
polyethylene tubing (PE-60, Clay-Adams) was inserted into
the cloaca and secured with a purse string ligature. At least
5 hr, and usually 10, were allowed between implantation of
the catheter and the start of an experiment. Catheterization
was employed in all cases where urine flow was measured or
where the concentration of a substance in the urine was re-
quired, in measuring sodium output, and in most of the
measurements of inulin clearance.

Blood sampling. Blood was obtained either by heart puncture
with a syringe and short 26-gauge needle (the animal
previously stunned by a blow on the head), or, after decapi-
tation, by collecting directly from the vescels of the severed
neck into a centrifuge tube. When an anticoagulant was
required, sodium heparin was used except in samples for
sodium analysis, where ammonium heparin or potassium
oxalate was employed. Ammonia and urea concentrations
were obtained by assay of whole blood; plasma was used in
all other analyses. When deproteinization of blood (or plasma)
was necessary, as in the determination of ammonia and
urea, an aliquot of the blood was added promptly to an
equal volume of cold 20% trichloroacetic acid. After stand-
ing 10 min on ice, the protein was removed by centrifuga-
tion. Trichloroacetic acid was eliminated by ether extrac-
tion prior to the determination of urea.

Injection and Infusion

Compounds for injection or infusion were dissolved in
distilled water except where otherwise indicated. Both injec-
tions and infusions were made into the doral subcutaneous
lymph space of the toad.

Analytical Methods

The concentration of "ammonia" (i.e., total ammonium
ion plus dissolved ammonia gas) was estimated by a micro-
diffusion-colorimetric technique. To assure specificity, am-
monia was isolated by alkalinizing the fluid to be analyzed
and collecting the evolved ammonia in citric acid (25).
Then the amount of ammonia collected was determined by
the "phenol-hypochlorite" reaction according to Chaney and
Marbach (7). Urea concentration was found by incu-
bating an aliquot of the fluid with urease after alkaliniza-
tion. The results of ammonia and urea analyses were ex-
pressed as microgram atomic weights of nitrogen per milli-
liter (atoms N/ml).

Sodium was determined by flame photometry using the
Coleman model 21 flame photometer. Standard sodium
solutions were read with each set of samples. The concentra-
tion of plasma protein was estimated according to Lowry,
Roebrough, Farr, and Randall (19). Values were expressed
in terms of a bovine serum albumin standard. Hematocrit
was measured by centrifugation of the blood in heparinized
capillary tubes for 5 min in a microhematocrit centrifuge.
The osmolality of fluids was determined with a Fiske
freezing-point osmometer.

Inulin was measured both by chemical methods and by
use of the carbon 14-labeled compound. In the few cases
where the unlabeled compound was used, inulin was deter-
mined by the direct resorcinol method of Roe, Epstein, and
Goldstein, as described by Smith (35); for these determina-
tions, plasma was deproteinized with cadmium sulfate.
In most of the clearance studies, inulin-carboxyl14C (aqueous,
5 µc/ml, New England Nuclear Corporation) was used and
the fluids were analyzed for 14C in a liquid scintillation spec-
trometer (Nuclear Chicago, Mark I). To compensate for
variable quenching, counting efficiency was monitored with
an automatic barium external standard by a channels ratio
method (calibrated with toluene-14C under several quench-
ing conditions and checked against an inulin solution of
known activity). The scintillation fluid was Bray solution
(6). Thixotropic gel powder (35 g, Cab-O-Sil, Packard)
was added to each liter of scintillation fluid; this led to more
reproducible count rates, especially when plasma was being
counted. The volume of sample counted was 0.1-0.25 ml for
plasma and less than 0.5 ml for urine or aqueous solutions.
15 ml of scintillation medium were added to each sample.

Measurement of Glomerular Filtration Rate

The glomerular filtration rate (GFR) of Xenopus was
measured by inulin clearance (34). The renal clearance of a
substance is defined as $C = UV/P$ (where $U =$ concentra-
tion in urine, $V =$ urine flow, $P =$ concentration in plasma).
To avoid any effects of handling and blood sampling on
renal function, we employed a single-injection clearance
 technique which required only one blood sample, at the
end of the experiment. This method takes advantage of the
inverse relationship between the clearance of a substance
and the "fractional time" for its excretion (see ref. 34). Ass-
suming a compound is distributed in the body fluids as if it
were in a single "compartment", then its clearance is given

$C = \frac{Q}{\text{Fractional time}}$

where $Q$ is the rate of infusion and $\text{Fractional time}$ is the
portion of the experiment when the compound was present in
the body fluids. When the compound is removed from the
body fluids, the clearance is zero.

To determine the fractional time, we measured the concen-
tration of inulin in plasma prior to and after injection of
inulin-carboxyl14C. The fractional time was calculated by
plotting the logarithm of the ratio of inulin concentration
at time $t$ to inulin concentration at time 0 against time.
The fractional time was the time at which the ratio was
reduced to 1, which corresponds to the time at which
99.8% of the inulin was removed from the body fluids.

The glomerular filtration rate was calculated by

$C = \frac{Q}{\text{Fractional time}}$
by
\[ C = \frac{(S \ln 2)}{t_{1/2}}, \]
where \( C \) = clearance, \( S \) = volume of distribution (inulin space, in this case), \( t_{1/2} \) = half-time for excretion. The derivation of this equation and details of the experimental method are given by McBean (21). In brief, the amount of inulin remaining in a toad at several times after injection was computed by subtracting the total amount excreted at a particular time from the amount originally injected. The inulin space was estimated from analysis of a terminal blood sample. Inulin-\(^{14}\)C, 3-4 \( \mu \)c, in a total volume of 0.8-0.9 ml were administered per 30 g. The dose of inulin solution was measured precisely and was injected with great care to avoid loss; if material leaked from the puncture site during injection, it was dispersed in the animal's medium, the volume of which was known. In uncatheterized animals, correction was made for the effects of leakage by subtracting from the amount injected the amount of isotope appearing in the medium during an equilibration period of 1.5 hr or more after injection. In catheterized toads, where operative trauma might cause leakage from the skin around the cloaca, water samples were taken after equilibration and at one or two additional times during the experiment. Leakage was usually negligible; if it exceeded 10% of the amount administered, the animal was rejected. Correction was also made for catheter dead space error.

This method yielded estimates of glomerular filtration rates (in freshwater toads) and inulin space (extracellular fluid volumes) comparable to those found by conventional methods in other species of amphibians maintained in freshwater (19, 28). Furthermore, inulin clearances determined by this method were similar in catheterized and uncatheterized toads. Catheterization was adopted for a majority of determinations in order to enable simultaneous measurement of urine flow.

Mathematical Examination of Effect of Changes in Urine Flow on Blood Urea Concentration

In order to evaluate the contribution of changes in urine flow to the increases in blood urea concentration observed during the adaptation of *Xenopus* to saline, an equation was derived from which plasma urea level could be calculated as a function of both urine flow and rate of urea synthesis, with these considered as functions of time. The derivation and application of the equation is described elsewhere (21). In summary, because the urine of *Xenopus* has the same concentration of urea as does the blood (3), the rate of change of blood urea concentration is given by

\[ \frac{dP}{dt}(t) = \frac{\Delta(t)}{S} - \frac{V(t)}{S} P(t), \]

where \( P \) = concentration of urea in blood, \( A \) = net rate of urea synthesis, \( V \) = urine flow, \( S \) = urea space. Several times during adaptation, the rate of urea synthesis, \( \Lambda \), was estimated indirectly by adding the rate of excretion of urea at a given time (Fig. 2) to the rate of change in body urea content at that time; the latter was taken to be the product of the urea space and the rate of change in blood urea concentration (Fig. 1). On the basis of data from other species (see ref. 21), the urea space (5) was assumed to be 60% of the body weight. The calculated values for \( A \) are published elsewhere (21, 23). Thus, starting with a known value of \( P \) at \( t = 0 \) (e.g., at the time of transfer to saline), then entering successive experimental values for \( V \) and \( A \) (21), and integrating numerically (by computer), one can estimate the blood urea level at various times after transfer of toads to saline. Most important, by holding constant (at the freshwater level) either the value for synthesis or the value for urine flow, it is possible to observe how these two variables interact in affecting the blood urea level. Initial computations indicated a discrepancy between the experimentally observed concentrations of urea in the blood and those calculated from the model. This discrepancy suggested that the urine flow was higher than normal in the catheterized toads in which flow was measured, perhaps because the urine was allowed to bypass the bladder (21). All urine flows were therefore multiplied by a single "correction factor." The results of computations with adjusted urine flow data are shown in Fig. 4.

RESULTS

When *Xenopus* toads were kept in saline solution for 2-3 weeks (average 2.5 weeks) a pronounced uremia developed (Table 1) as observed in previous studies (5, 15, 22). At the end of the experimental period, blood urea had increased 15-fold (compared to freshwater controls), whereas blood ammonia concentration was at freshwater levels (Table 1). Immediately after the transfer of the toads to saline, blood urea began to increase rapidly (Fig. 1). By 2 days, the urea concentration was 4 times that of 0 day freshwater toads and by 15 days, it had reached its maximum level. The concentration of ammonia, on the other hand, showed no significant increase at 2 days. By 2 weeks, it had declined to slightly below freshwater levels, where it remained. The
TABLE 1. Effect of a hyperosmotic environment on ammonia and urea metabolism in Xenopus laevis

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood N Concentration, μatoms/ml</th>
<th>Nitrogen Excretion, μatoms/100 g per hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ammonia</td>
<td>Urea</td>
</tr>
<tr>
<td>Freshwater</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30±0.03</td>
<td>0.9±1.2</td>
</tr>
<tr>
<td>Saline</td>
<td>0.27±0.04</td>
<td>105.0±6.7</td>
</tr>
<tr>
<td></td>
<td>(P&lt;0.01)</td>
<td>(P&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>24±2.6</td>
<td>9.5±2.5</td>
</tr>
<tr>
<td></td>
<td>(1.05&lt;P&lt;1)</td>
<td>(1&lt;P&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>33±4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ratio of saline to freshwater values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

Toads were fasted for 18 days in freshwater. They were then divided into two groups of 8 toads each. One group was kept in freshwater for an additional 2-3 weeks (average 2.5 weeks). The other was kept in saline (300 mOsm/liter) for the same period of time. Excretion rates and blood values were measured on the last day of the experiment. Values are means ± se. Probabilities are given in parentheses.

![Graph of excretion of ammonia and urea by Xenopus laevis during a 3-week period in saline.](image)

Concentration of sodium in the plasma rose promptly upon transfer of the animals from freshwater to saline. Within the first few days in saline, a maximum of more than 130% of control was reached. By 1 week after transfer, however, plasma sodium had returned to a level 15% above the concentration in freshwater toads.

There was an immediate reduction in the excretion of both ammonia and urea after Xenopus toads were transferred to saline solution (Fig. 2). The excretion of both compounds remained at minimum levels for about 2 days and then began to increase again. By 7 days the excretion of ammonia had reached an average rate of about three-fourths that in freshwater (and not significantly different from the freshwater level), where it remained for the duration of the experimental period. Urea output, on the other hand, continued to rise until 2 weeks after transfer; then it stabilized.

The rate of excretion of urea in toads kept in saline for 2–3 weeks was 4–5 times that of freshwater controls (Table 1). In contrast, the output of ammonia was not significantly changed, although it was, on the average, lower than in the freshwater controls (Table 1). A stable condition, indicating the completion of adaptation, was reached during the 2nd week in saline; both blood levels and excretion rates of urea and ammonia remained at fairly constant levels after the 2-week point (Fig. 1 and Fig. 2). Therefore, the blood nitrogen concentrations and excretion rates presented in Table 1 (for toads 2–3 weeks after transfer) should be close to steady-state values. In addition, two animals were examined at times beyond 3 weeks. These toads had been in saline for 2 and 6 months, respectively, and had been fed occasionally during those periods to avoid starvation. Blood urea concentrations were 80 and 91 μatoms N/ml, very close to the mean values for toads kept in saline only 2–3 weeks (cf. Table 1). Likewise, the rates of excretion of ammonia and urea were close to those found in 2–3 week toads. These observations suggest that the adapted state persists indefinitely in a form similar to that seen during the 3rd week after transfer to saline.

Urine flow and glomerular filtration rate were measured during the adaptation of Xenopus to saline to determine if there were any changes in renal function which could be related to changes in the blood concentrations and excretion rates of urea and ammonia. A dramatic decline in urine flow was observed (Fig. 3) within a few hours after the medium of a toad was changed from freshwater to saline solution. On the average, urine production was reduced to 8.4 ± 3.7% of control levels (five animals). The mean time for the new relatively steady rate to be reached was 2.6 hr after changing the medium. After a week in saline, urine flow had returned...
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to about 30% of the freshwater level (Fig. 3); it did not change significantly from this time to the end of the 3-week experimental period. To estimate how much the observed increase in blood urea level was the result of the pattern of urine flow changes just described, hypothetical blood urea levels were computed by means of the equation given earlier (METHODS) which relates changes in urea concentration in the body fluids to changes in urine flow and/or urea synthesis. The results of this procedure (Fig. 4) suggest that a decrease in urine flow is essential to the elevation of plasma urea during the adaptation of Xenopus to saline. The effects of changes in urine flow and synthesis are not simply additive, but are synergistic; in other words, the lowered urine flow increases the effect of increased synthesis more than would be predicted from simple addition of the two effects. Without a decreased urine flow, the increased synthesis could not raise the plasma urea level above curve A, which, at 21 days for example, represents less than 30% of the actual concentration. Thus, while it cannot be called the predominant cause of increased plasma urea concentration, the decrease in urine flow is very important.

Glomerular filtration rates (measured simultaneously) followed the same pattern as urine flow (Fig. 3), a fact which suggested that the changes in urine flow were to a large extent due to changes in GFR. It was not practicable to measure GFR accurately during the period of acute reduction of urine flow, but from the correlation between GFR and urine flow at other times, it seems likely that the very low urine flows measured after changing the medium to saline were largely the result of correspondingly low filtration rates.

Urinary osmolality was elevated after the toads had been in saline solution for 3 weeks, but the urine remained hypertonic to the blood (Table 2). Likewise, the concentration of urea in the urine was higher after 2–3 weeks in saline (82 ± 11 μatoms N/ml, six animals, various fasting periods) than in freshwater (6.5 ± 1.5 μatoms N/ml, eight animals) apparently reflecting the increase in plasma level. Urine ammonia concentration was also increased in saline (16 ± 2.7 μatoms N/ml for six animals versus 8.5 ± 0.75 μatoms/ ml for eight animals in freshwater) as was urine sodium (32 ± 5.9 mEq/liter for three animals versus 9.7 ± 2.0 mEq/liter for seven animals in freshwater). However, the increases in both ammonia and sodium concentrations were offset by the reduced urine flow so that the output in saline was no greater than in freshwater.

It seemed likely that the stimulus for a reduction in urine flow would be either a change in the volume of one of the body fluid compartments (presumably either the extracellular or the intravascular) or else an increase in the sodium concentration in the extracellular fluid. Accordingly, these variables were examined at several times after transfer to saline. The inulin space of an animal approximates the volume of its extracellular fluid, and hematocrit and plasma protein concentration both tend to change inversely with the volume of the vascular compartment (30). No change was seen in any of these indices at any time except for a slight fall in hematocrit at 2 days, suggesting a temporary dilution of the blood (Fig. 5). In addition, the muscle inulin space of the two toads in saline (10 days) averaged 14%, normal for hydrated amphibians (12). Some loss of weight did occur during the first day in saline; while toads kept in

FIG. 5. Inulin space, hematocrit and plasma protein concentration in Xenopus laevis during adaptation to saline. Each point is mean of values from following numbers of animals: inulin space: 0 days, 7; 8 days, 6; 16 days, 4; 21 days, 4; plasma protein: 0 days, 7; 2 days, 7; 7 days, 5; 21 days, 4; hematocrit: 0 days, 6; 2 days, 4; 7 days, 6; 21 days, 4. Vertical lines above and below means represent SE.
freshwater showed insignificant changes in weight (+0.65 ± 0.39\% of body weight for four control toads weighed before and after 4 hr in freshwater), toads placed in saline had lost 3.9 ± 0.3\% (four animals) after 4 hr and about 10\% after 12 hr. However, at the end of the 3-week period, toads in saline showed no greater weight loss than their freshwater counterparts (12 ± 2.3\% for four animals in saline versus 11 ± 2.6\% for four animals in freshwater). Therefore, dehydration per se did not appear to occur during saline adaptation except in the very early stages. There was, however, an elevated extracellular sodium concentration in toads placed in saline (Fig. 1).

A rapid increase of plasma sodium concentration started soon after the toads were transferred to saline. The elevation was probably significant within the 1- to 2-hr period that usually elapsed before the onset of severely reduced urine flow since the two toads sampled at about 4 hr after transfer had plasma sodium values of 120 and 125 mEq/liter, compared to a mean for eight freshwater animals of 101 + 4.8. Very likely the rapidity of the rise in sodium concentration was due to the sudden exposure of epithelial active transport systems to an increased external concentration, with some contribution also from loss of body water. Within two days (Fig. 1) plasma sodium averaged 30\% above the freshwater level. After the first few days, plasma sodium declined but never returned to normal (Fig. 1). The mean value for plasma sodium concentration at 21 days (125 mEq/liter, Fig. 1) may not be as representative as the values for 0, 2, and 7 days, since two freshwater controls (treated in parallel with toads of the 21-day saline group and killed at the same time) had much higher plasma sodium concentrations (mean, 119 mEq/liter) than the rest of the toads in the freshwater group (mean, 96 mEq/liter). Increases in the concentrations of both sodium and urea aided in the toads' achieving osmotic equilibrium with the otherwise hyperosmotic environment, (Table 2). At first, the elevation in osmotic pressure of the plasma was due mostly to sodium, but by 7 days (and thereafter) urea contributed the major fraction of the increase. Thus, the accumulation of urea permits osmotic balance to be maintained without excessive increases in extracellular sodium concentration.

In view of the temporal relationship between the rise in plasma sodium and the decline in urine flow in toads placed in saline solution, the effect on renal function of increasing extracellular sodium concentration was studied by infusing sodium chloride in an amount calculated to raise the extracellular concentration by 30\%, the increment observed in 2-day toads. In all three animals tested, such an infusion had the same effect as placing toads in saline, i.e., to cause a rapid fall in urine flow (Fig. 6). Infusion of sucrose or glucose in amounts osmotically similar to the dose of sodium chloride (Fig. 6) did not cause urine flow to diminish. These compounds were tested in one and two animals, respectively, and their failure to elicit a response suggests that the effect is specific for sodium.

Limited investigations were made on the possible roles of nervous and hormonal systems in the antidiuretic response of *Xenopus* to osmotic stress. In the doses employed, neither the ganglionic blocker, hexamethonium chloride (45-600 mg/100 g, four toads), nor either of the peripheral agents, atropine sulfate (parasympatholytic, 150 μg/100 g, one toad), or phenoxybenzamine hydrochloride (sympatholytic, 0.3 mg/100 g, one toad) reversed the antidiuretic response to saline. Hexamethonium chloride (600 mg/100 g, two toads) did not cause reduction of the urine flow in freshwater. Pretreatment with methyrapone ditartrate (9 mg/100 g at 39 and 15 hr before transfer), an inhibitor of adrenal steroid biosynthesis, did not seem to affect either the urine flow in freshwater, or the antidiuresis after transfer to saline (two toads). In addition, the analysis of two samples of pooled plasma, one sample from animals in freshwater and one from animals in saline (2.5 weeks), showed little difference in the corticosterone content (0.45 μg/ml and 0.53 μg/ml, respectively).1 Angiotensin amide (18 μg/ml, plus 6 μg/100 g X hr) suppressed urine flow in the one freshwater toad to which it was administered. Arginine vasotocin (AVT) produced weak antidiuresis in two of four toads. One of these toads had been in saline solution for less than a day and not much further decrease in urine flow would be expected. In the freshwater toad (which received 2.6 μg/100 g, plus 0.7 μg/100 g X hr) the response was very much slower in onset than the normal response to saline, or than the response of *Rana pipiens* to lower doses of hormone (the latter occurs within 10 or 15 min after injection). In one freshwater

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1 Fluorometric assay of 11-OH corticosteroids, estrogens removed by alkaline wash, courtesy of Dr. W. J. Reddy.
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toad (1 μg/100 g) AVT had no effect, and in one saline toad (3 μg/100 g) there was an anomalous increase in urine production.

DISCUSSION

Xenopus shares, with some elasmobranchs and a number of other amphibians (10, 12, 13), the ability to adapt to changes in ambient salinity by altering the levels of retained solutes, especially urea. Urea accumulation will be promoted by reduced urine flow (since this lowers the urea clearance), especially in forms such as Xenopus in which there seems to be no active transport of urea. A sharp reduction in urine flow occurs on transfer of Xenopus from freshwater to saline solution. Such an antidiuresis in response to elevated salinity seems to be the rule in euryhaline lower vertebrates. For example, in the several species of euryhaline fish studied to date, urine flow and glomerular filtration rate decline within a few hours after transfer from freshwater to a medium of higher salinity (18). In amphibians, antidiuresis follows both dehydration and transfer to hyperosmotic solutions (1, 29). Because the kidneys of most lower vertebrates cannot form a hypertonic urine, GFR must be reduced under dehydrating conditions if severe renal water loss is to be avoided. On the other hand, in amphibians such as Xenopus which lack effective extrarenal excretory systems, it would appear that a drastically reduced GFR could not be sustained indefinitely without an accumulation of waste products to hazardous levels. For extended survival of such species in a hyperosmotic environment the only course seems to lie in attaining hyperosmolality by accumulation of a rapidly synthesized, nontoxic compound so that adequate renal excretion can be resumed. The pattern of events during the adaptation of Xenopus appears to conform to this. As osmotic balance improves (because of the accumulation of urea), urine flow increases; it always remains far below the rate in freshwater, however, thus continuing to promote the elevated concentration of urea in the body fluids.

Of the physiological variables monitored, the sodium concentration in extracellular fluid appeared to be the only one related to antidiuresis. The largest increases in plasma sodium concentration during saline adaptation of Xenopus, i.e., around 30% above control, do not seem to be out of the range encountered in the amphibian world—similar increments are seen in the semiaquatic toad, Bufo marinus, after moderate dehydration (31), and in the desert toad, Scaphiopus couchii, after natural hibernation in the soil (24). Antidiuresis after administration of hyperosmotic sodium chloride has been observed in several lower vertebrates. In B. marinus (a species which also reduces its urine flow when placed in hyperosmotic saline) a single injection of 1.5 mEq/100 g (enough to increase extracellular sodium by at most 40%) causes an antidiuresis identical to that induced by loss of 12–20% of the body weight through dehydration (31, 32). Reduced urine flow after saline loading also has been reported in turtles and a few other reptiles (9, 26). Thus, Xenopus is not alone in its antidiuretic reaction to injection of hyperosmotic salt solution; extracellular sodium concentration may be of widespread importance in the control of urine flow in lower vertebrates, especially in those species (fish, urodeles) which, like Xenopus, are not very sensitive to the so-called antidiuretic hormone, arginine vasotocin. The failure of sucrose and glucose loads to induce antidiuresis in Xenopus suggests that the renal response to saline is not simply a reaction to increased osmotic pressure.

The system linking increased extracellular sodium concentration with a reduction in GFR remains obscure. Adolph (2) noted that after surgical denervation of the kidneys, frogs still responded to transfer to a hyperosmotic solution by reducing urine flow. The brief pharmacological studies described in the present paper tend to confirm his suggestion that the nervous system has no direct part in this response. Chester-Jones, Chan, and Rankin (8) have found that arterial (dorsal aorta) blood pressure averages about 25% lower in seawater-adapted eels than in freshwater ones. In amphibians such a pressure drop would probably be sufficient to cause the net filtration pressure to approach the colloid osmotic pressure of the blood (see, for example, ref. 38) and thus to cause a marked depression of GFR. Failure of hexamethonium to modify the antidiuretic response of Xenopus after transfer to saline suggests that if, in this species, the antidiuresis were a result of a fall in blood pressure, then the latter would be a mechanical response of an uncompensated system to loss of water. In view of the apparent absence of dehydration during saline adaptation such an explanation seems unlikely. Moreover, the reduction of urine flow in Xenopus during saline infusion suggests a regulatory mechanism other than blood pressure, at least in the acute antidiuretic response.

Xenopus proved to be quite refractory to the antidiuretic property of arginine vasotocin. This is in accord with the failure of other investigators to observe weight gain in Xenopus after treatment with this hormone (14). On the other hand, it is conceivable that the simultaneous release of more than one hormone is required to produce the antidiuretic effect in Xenopus. Alternatively, since AVT is antidiuretic in the more terrestrial amphibians, but diuretic in fish (27), it is possible that it has an intermediate role in the aquatic amphibia, viz, that it has little or no direct effect on urine flow and is involved only in the regulation of sodium balance. The inconsistent and mostly small changes in urine flow after administration of AVT to Xenopus thus might depend on the influence of AVT on plasma sodium levels. At any rate, it seems doubtful that arginine vasotocin alone is the mediator of the antidiuretic response of Xenopus to saline.

There are some suggestions, from comparative studies, that renin production and GFR may vary together. For example, saltwater fish tend to have a lower kidney renin content and a lower GFR than freshwater ones (36); however, such a correlation seems hard to confirm statistically during the adaptation of a given species to a new salinity (20). According to Johnston et al. (17), the kidneys of the bullfrog contain a pressor substance similar or identical to renin and apparently associated with juxtaglomerular cell granules. As in mammals, these granules are more prominent under conditions of low salt availability (e.g., immersion in freshwater) than in situations promoting hypernatremia (saline media or dehydration). In the rat, it has been claimed that elevation of the sodium concentration in distal tubular fluid (at the macula densa) may bring about
a reduction in GFR and that such a response is dependent on the concentration of renin in the juxtaglomerular cells (37). It is conceivable that the “water-conserving” antidiuresis seen in *Xenopus* and other lower vertebrates in response to elevated plasma sodium is the primitive homologue to the “salt-conserving” relation between filtration and early distal sodium concentration seen in the rat. One hypothesis is that, at physiological levels, angiotensin causes constriction mainly of the postglomerular arteriole thus promoting filtration (36); at a high dose (as in the present study), both arterioles would be strongly affected and filtration reduced. From a comparative viewpoint, however, recent observations in elasmobranchs seems to indicate that angiotensin may not have a major role in the control of GFR in lower vertebrates; the skate (*Raja erinacea*), in which the presence of a renin-angiotensin system is doubtful (36), still responds to a change in salinity with change in GFR (11). Thus, the mediator of the antidiuretic response of *Xenopus* to saline stress remains in doubt. Whatever the mediator, the failure of autonomic blocking agents and the levels of circulating hormones clearly to influence urine flow in *Xenopus* makes an intrarenal mechanism appear likely.

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