Renal function in response to reduced osmotic load in larval salamanders

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STIFFLER, Daniel F., and Ronald H. ALVARADO. Renal function in response to reduced osmotic load in larval salamanders. Am. J. Physiol. 226(5): 1243-1249. 1974.-Renal function was studied in larval Ambystoma gracile and Dicamptodon enatus exposed to several osmolarities. Clearance methods were employed in free-swimming larvae using inulin as a GFR marker. GFR, as calculated from the rate of appearance of inulin in the bath, declined from 0.113 to 0.023 ml/10 g-h (A. gracile) and from 0.166 to 0.006 ml/10 g-h (D. enatus) between 2 mosM (tap water) and 300 mosM (sucrose). In experiments involving urine collection (A. gracile), urine flow decreased from 0.078 in controls to 0.018 ml/10 g-h in the 200-mosM group while GFR decreased from 0.104 to 0.028 ml/10 g-h. The latency for the reduction in GFR is about 2 h; however, the return to normal GFR after return to tap water takes about 6 h. Fractional reabsorption of water increased from 25 to 37%, in the 200-mosM group, while fractional reabsorption of Na+, K+, and Cl− decreased slightly. The results indicate that both glomerular and tubular factors (and possibly the bladder or cloaca) are involved in the response to reduction in osmotic load; however, the decrease in GFR predominates.

METHODS

Animals. Larvae of two ambystomatid salamanders were studied. Ambystoma gracile were trapped in Owl Creek, near Corvallis, Oregon; Dicamptodon enatus were taken by hand from Parker Creek on Mary’s Peak in Benton County, Oregon. The animals were maintained at 15 ± 1°C in dechlorinated tap water and remained in the laboratory for at least 1 wk before use. Some of the animals were fed raw beef heart; feeding was suspended 2 wk before use of the animals. Anesthetic. When anesthesia was required, the animals were immersed in 0.1% tricaine methanesulphonate with the pH adjusted to neutrality with NaHCO3. Anesthesia was complete within 5 min for D. enatus and 20 min for A. gracile.

Chemical analyses. Urine and plasma which could not be analyzed immediately were stored at -10°C. This had no effect on the substances assayed. Sodium and potassium concentrations were measured by flame photometry (precision ± 1%) after appropriate dilution. Chloride was titrated with a Cotlove chloridometer (precision ± 1%). Undiluted urine and plasma samples were analyzed for total solute concentration with a vapor-pressure osmometer (precision ± 2%).

Assay of radioactivity. Carbon 14 was counted in a liquid scintillation spectrometer. Corrections for quenching were made by the channels ratio method. In one series of experiments the radioactivity in the bath containing animals previously injected with 22Na was monitored continuously by pumping the water through a coil located in the well of a NaI crystal. This detector was used in conjunction with a rate meter connected to a potentiometric recorder.

Calculation of glomerular filtration rate from rate of inulin excretion into bath. In aquatic animals which do not drink and which excrete inulin through the kidney only, the glomerular filtration rate (GFR) is the ratio of the rate of loss of
inulin to the bath divided by the mean plasma concentration of inulin during the experimental period (2, 10, 12, 16).

Obtaining a suitable estimate of the mean plasma inulin concentration is complicated by the fact that plasma concentration decreases with time because of excretion by the kidneys. This difficulty has been overcome by using short clearance periods so that the decrease in plasma inulin is relatively small and the inulin concentration of a terminal blood sample can be used directly (2, 16). Another approach has been to take a blood sample at the onset and end of the clearance period by cardiac puncture (10, 12). The use of the first method would be unreliable in our study because of the rather long clearance periods. We avoided the second method because the animals were small (ca. 10 g) and removal of an adequate amount of blood might have significantly reduced their blood volume. More important, cardiac puncture might damage the heart sufficiently to alter hemodynamics and thus affect renal filtration pressure. We developed a method which estimates the initial plasma inulin concentration from the analysis of a terminal plasma sample, the inulin space, and the rate of renal excretion of inulin.

A known amount of inulin [COOH-14C]inulin, New England Nuclear) was injected subcutaneously into several animals. After specified intervals they were killed and the inulin space was calculated as:

\[ S = \frac{T - E}{P} \]  

where: \( S \) = the inulin space, ml; \( T \) = total inulin injected, disintegrations per minute (dpm); \( E \) = inulin excreted, dpm; \( P \) = plasma inulin concentration, dpm/ml. A regression line of inulin space as a function of time was then computed for each species.

The mean plasma concentration of inulin during the clearance period was calculated from the relationship:

\[ \frac{(P_f)(S_0) + (E)}{S_0} \frac{1}{2} P_t \]  

where: \( P \) = mean plasma inulin concentration during the clearance period, dpm/ml; \( P_t \) = plasma inulin concentration at the end of the clearance period, dpm/ml; \( S_0 \) = inulin space at the beginning of the clearance period, ml; \( S_t \) = inulin space at the end of the clearance period, ml; \( E \) = amount of inulin excreted during the clearance period, dpm. The calculated mean plasma inulin concentration was usually 10-20% higher than the terminal plasma inulin concentration. The method assumes that, following a single injection, the concentration difference for inulin between the interstitial fluid and plasma is small compared with the concentration in each compartment (19).

The GFR of unanesthetized, unrestrained animals was estimated by the "free-flow" method described above. Animals were injected subcutaneously with [COOH-14C] inulin (1 μCi) and after an appropriate equilibration period (12 h for \( A. \) gracile and 18 h for \( D. \) ensatus) they were placed in individual containers with the appropriate solution. Samples of the bath were assayed for radioactivity at regular intervals and the GFR was calculated from:

\[ \text{GFR} = \frac{d\ln/dt}{P} \]  

where: \( d\ln/dt \) is the rate of appearance of inulin in the bath. Values were adjusted to a standard of 10 g of body weight.

Renal tubular function. A second means of assessing renal function involved direct collection of urine. Salamanders that had been previously injected with inulin were anesthetized and their bladders emptied by gentle suprapubic compression. Urine was collected in bags prepared in the following way. A clean condom with the tip cut out was drawn over the head and forelimbs of each animal and tied in place anterior to the pelvic girdle. Another hole was made so that the tail of the animal could pass out of the bag. The bag was then sealed around the tail with a second ligature. At this point fluid from the cloaca would drain into the bag located ventral to the cloaca. The bag was closed with a third ligature and the animal was placed in the bath. Animals placed in 200 mosM sucrose were allowed to equilibrate for 4 h before attaching the bags. After 6-12 h, the urine in the bags was transferred to a tared vial and weighed. A plasma sample was taken from each animal. The bath was monitored for radioactivity to detect leakage of urine from the bags.

To determine the extent of urinary water absorption by the skin, bags were tied to the midsections of animals in such a way that their cloacae did not open into the bags. A solution containing the same total solute concentration as urine (20 mosM sucrose) and a known concentration of inulin was then placed in the bag. The ratio of final (12 h) inulin concentration to initial inulin concentration was 0.98 ± 0.03 (standard error of the mean (SEM)) indicating little absorption.

Statistics. Data are expressed as the mean and the SEM.
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Fig. 2. Inulin space as a function of time in A. gracile (---) and D. ensatus (---). Each point is a single animal.

TABLE 1. Glomerular filtration rate of larval salamanders immersed in tap water

<table>
<thead>
<tr>
<th>Mean Wt, g</th>
<th>GFR, ml/10 g-h</th>
<th>Winter</th>
<th>Spring-fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. gracile</td>
<td>11.7</td>
<td>0.035</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>±0.5</td>
<td>±0.016 (5)</td>
<td>±0.012 (15)*</td>
</tr>
<tr>
<td>D. ensatus</td>
<td>12.3</td>
<td>0.166</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.9</td>
<td>±0.019 (9)†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM according to the season during which the measurements were made. The temperature was 15°C. Figures in parentheses are numbers of animals. * Significantly higher than winter value (P < 0.025). † Significantly higher than spring-fall value of A. gracile (P < 0.05).

RESULTS

Frequency of urination. To determine if retention of urine in the bladder is significant the frequency of urination was studied in four larval and one adult A. gracile. Twenty-four hours after injection with 2 μCi of sodium 22 we transferred each animal to 25 ml of tap water and monitored the rate of appearance of 22Na in the bath continuously. Representative results from one larva and the adult are given in Fig. 1. The curve relating 22Na activity to time was nearly linear in larvae reflecting little storage of urine. In contrast, the stepwise pattern of the adult suggests storage of urine in their bladders with intermittent urination.

Inulin space. Figure 2 shows the change in inulin spaces with time in A. gracile and D. ensatus. The inulin space declined at first, reached a minimum at 6-18 h, and then slowly increased. The time required to reach the minimum space is the time to reach uniform distribution of inulin, about 12 h for A. gracile and 18 h for D. ensatus. Between 18 and 60 h the inulin space of D. ensatus increased at the rate of 0.021 ml/10 g-h, whereas the inulin space of A. gracile increased at the rate of 0.016 ml/10 g-h between 12 and 30 h and then remained essentially constant for the next 30 h. Extrapolating these curves to zero time gives an estimate of extracellular volume (24) which is 2.3 ml/10 g for D. ensatus and 3.2 ml/10 g for A. gracile. Published values for extracellular volume in urodeles (sucrose space) range from 2.18 ml/10 g in Amphiuma means tridactylum to 2.41 ml/10 g in Necturus maculosus (24). Our value for A. gracile is high for reasons which are unknown. We could regularly obtain larger blood samples from A. gracile than from D. ensatus of comparable size.

Free-flow determination of GFR. Table 1 shows the GFR values for larvae in tap water under free-flow conditions. Seasonal and species differences are evident. The GFR of A. gracile in the winter was about 50% of the values measured in other parts of the year (P < 0.025). The GFR for "spring-fall" D. ensatus is higher than for spring-fall A. gracile (P < 0.05).

Figure 3 illustrates the effect of external osmolality on GFR in the two species. The A. gracile investigated during spring through fall decrease their GFR linearly over the range of 2-300 mosM sucrose. Winter A. gracile have a depressed GFR in tap water which does not fall lower until the medium becomes hyperosmotic (300 mosM). D. ensatus (spring-fall) also show a decrease in GFR as the osmolality of the bathing solution rises. However, the decrease is more precipitous than in A. gracile up to the isosmotic concentration (about 200 mosM, see Table 3), and then levels off between 200 and 300 mosM.

The decrease in GFR occurs a few hours after the increase in external osmolality. To better establish the duration of this latency the bath was sampled at short intervals after...
removed the bags and checked for urine after 6, 12, and 18 h (separate animals). The accumulation of urine was linear with time, yielding a slope of 0.064 ml/10 g·h and an intercept of 0.10 ml/10 g. The y intercept was not significantly different from zero (P > 0.5). The bags remained in place for 12 h or less in the experiments reported below.

The collection and analysis of urine from the bags enabled us to evaluate tubular and glomerular components of excretion. Since the urine was collected from the cloaca and not from the ureters, it is possible that it was modified by the bladder and/or the cloaca, especially during low urine flow. Although this possibility cannot be ruled out, we have not observed unusually high inulin U/P values when urine was forced to accumulate in the bladders of larvae in tap water or 200 mosM sucrose (23).

Table 2 summarizes the data from *A. gracile* measured during the spring. The filtration rates agree closely with those obtained by the free-flow method (Table 1). Animals in 200 mosM sucrose reduced their GFR and increased their fractional reabsorption of water (as indicated by increased inulin U/P values; see also Table 4). Consequently, both urine flow and fractional water excretion decreased.

Urine and plasma solute concentrations appear in Table 3. The osmolality and Na⁺, K⁺, Cl⁻ concentrations of urine increased two- to threefold after the animals were challenged with 200 mosM sucrose solution. Plasma Na⁺ and Cl⁻ concentrations remained essentially constant. The apparent higher K⁺ concentration in plasma from animals in

### Table 2. Renal function in Ambystoma gracile larvae in tap water and 200 mosM sucrose

<table>
<thead>
<tr>
<th></th>
<th>V (U/P)</th>
<th>GFR</th>
<th>C₂O₃-M</th>
<th>CH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>0.078</td>
<td>1.3</td>
<td>0.104</td>
<td>0.014</td>
</tr>
<tr>
<td>±0.006</td>
<td>±0.1</td>
<td>±0.007</td>
<td>±0.001</td>
<td>±0.006</td>
</tr>
<tr>
<td>Tap water</td>
<td>0.00</td>
<td>1.6</td>
<td>0.029</td>
<td>0.00</td>
</tr>
<tr>
<td>200 mosM sucrose</td>
<td>0.000</td>
<td>±0.1</td>
<td>±0.005</td>
<td>±0.000</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SEM; figures in parentheses are numbers of animals. V = rate of urine production; GFR = glomerular filtration rate; C₂O₃-M = osmolar clearance; CH₂O = free water clearance; all expressed as ml/10 g·h.

### Table 3. Composition of urine and plasma of Ambystoma gracile larvae

<table>
<thead>
<tr>
<th></th>
<th>Tap Water</th>
<th>200 mosM Sucrose</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total solute</td>
<td>37±2 (20)</td>
<td>86±8 (14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Na⁺, μeq/ml</td>
<td>10.2±0.7 (20)</td>
<td>26.9±3.5 (16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cl⁻, μeq/ml</td>
<td>6.4±0.4 (20)</td>
<td>17.4±2.0 (16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>K⁺, μeq/ml</td>
<td>3.9±0.4 (20)</td>
<td>6.2±0.8 (16)</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total solute</td>
<td>191±2 (27)</td>
<td>192±2 (15)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Na⁺, μeq/ml</td>
<td>94.4±2.4 (24)</td>
<td>91.1±1.5 (25)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Cl⁻, μeq/ml</td>
<td>73.4±1.7 (18)</td>
<td>72.5±0.8 (24)</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>K⁺, μeq/ml</td>
<td>9.5±0.6 (23)</td>
<td>7.5±0.3 (25)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SEM; figures in parentheses are numbers of animals.
Renal reabsorption of water and ions in *A. gracile*

<table>
<thead>
<tr>
<th></th>
<th>Tap water</th>
<th>200 mosM sucrose</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absolute Reabsorption</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2O, ml/10 g-h</td>
<td>0.026 ± 0.004 (20)</td>
<td>0.010 ± 0.000 (17)</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Na+, meq/10 g-h</td>
<td>9.4 ± 0.7 (20)</td>
<td>2.5 ± 0.3 (16)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Cl-, meq/10 g-h</td>
<td>7.4 ± 0.8 (16)</td>
<td>2.0 ± 0.3 (16)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>K+, meq/10 g-h</td>
<td>0.7 ± 0.1 (17)</td>
<td>0.1 ± 0.0 (16)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td><strong>Fractional Reabsorption, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap water</td>
<td>25 ± 4 (20)</td>
<td>37 ± 4 (17)</td>
<td>$&lt;0.03$</td>
</tr>
<tr>
<td>200 mosM sucrose</td>
<td>92 ± 1 (20)</td>
<td>85 ± 2 (16)</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

Values are means ± SEM; figures in parentheses are numbers of animals. Absolute reabsorption refers to the amount filtered less the amount excreted. Fractional reabsorption refers to the absolute reabsorption divided by the amount filtered expressed as a percent.

**DISCUSSION**

Previously published values for the GFR of larval urodèles measured by the free-flow method range from 0.08 (12) to 0.33 ml/10 g-h (16). Our values for spring-fall larvae of *A. gracile* and *D. ensatus* fall between these extremes. Discrepancies reflect species, methodological, and seasonal differences in the studies.

We observed species differences. The GFR of *A. gracile* is significantly lower than that of *D. ensatus* for animals of similar weight and measured under comparable conditions.

Methodological differences probably account for the major share of the differences. The route of injection of inulin and the method of estimating plasma inulin concentration have varied in different studies. The higher rates (16) were obtained from animals injected intraperitoneally and using a terminal plasma concentration. In experiments preliminary to the current investigation we found that inulin introduced into the coelomic cavity of larval *A. gracile* exchanges relatively slowly with plasma. Even after 8 h the coelomic fluid was an order of magnitude higher in inulin-14C than plasma. In *Necturus* about 11% of the tubular fluid is derived directly from the coelomic fluid via the nephrostomes (29). This could explain the higher GFR values obtained with intraperitoneal injection of inulin. Subcutaneous injection of the inulin circumvents the problem. The low values reported for *A. tigrinum* were based on subcutaneous injection (12), however, an initial blood sample was taken by heart puncture which could produce abnormal results.

There is increasing evidence that various aspects of renal function change with season. We observed a highly significant reduction in GFR in the winter even though measurements were made at the same temperature through-out the year. Adult toads (*Bufo arenarum*) also have a lower GFR in winter (25). Curiously, in *Necturus*, reabsorption of water is higher in winter than in summer but the GFR is the same (5, 11).

In amphibians the GFR is apparently an important physiological variable. The most significant observation of this study is that the GFR can be changed relatively rapidly by simply altering the osmotic load on the animals. Adult *Xenopus laevis* also reduce their GFR when the osmotic load is reduced by addition of mannitol to the bath (6). However, the time course of the response was not given. Curiously, no change in GFR was observed in adult *Rana esculenta* on addition of electrolytes to the bath (14), and in *Rana cavericola* the GFR remains quite high in 200 mosM saline, however, urine flow is reduced by an increased tubular reabsorption of water (21). At least some anurans (15) decrease their GFR when they are immersed in saline solutions.

The overall GFR of amphibians is determined by the number of functional glomeruli (20), glomerular blood pressure, tubular pressure, and oncocytic pressure of plasma. The first two seem the likely points of control and both can be related to constriction or dilation of glomerular vessels as well as overall changes in arterial pressure. Several hormones may affect the diameter of glomerular vessels including arginine vasotocin (17), angiotensin II (22), and glomerulopressin (26).

**Posterior pituitary extracts constrict afferent arterioles in frogs** (17) and arginine vasotocin (AVT) reduces GFR in frogs (9), toads (27), and larval salamanders (2). In higher vertebrates the release of antidiuretic peptides from the hypothalamic-neurohypophysial complex is initiated by an increase in solute concentration in plasma (98), which is
consistent with what would be expected when the osmotic gradient between the body fluids and bathing medium of aquatic amphibians is decreased. Frogs adapted to 150 mM NaCl have more stainable neurosecretory material in the preoptic nucleus than frogs adapted to tap water (18). On the other hand, after hypophysectomy and lesions of the preoptic nucleus larval _A. tigrinum_ chronically adapted to 100 mM NaCl still had a low GFR (10). Clearly further work is needed to clarify this point.

Angiotensin II constrains the efferent arteriole of frogs. Plasma renin activity, which should relate directly to angiotensin II levels in the blood, is higher in hydrated frogs than in dehydrated frogs (22). Since plasma renin activity is present under normal hydrated conditions, it is possible that the activity decreases when animals are placed in isosmotic solutions which would decrease the GFR.

In loads glomerulopressin increases glomerular pressure without affecting arterial pressure by causing dilation of the afferent arterioles (26). Plasma volume expansion produced by injection of 4% dextran produced similar effects suggesting that this may serve as a stimulus for the release of the hormone. Conceivably a decrease in plasma volume would produce the opposite effect and thus reduce GFR.

The GFR of _A. gracile_ and _D. ensatus_ decreased within 2 h after reducing the osmotic load. The nature of the stimulus which initiates this response and how it is detected remain unknown. By analogy with what is found in mammals, one might expect sensitive internal osmoreceptors which respond to changes in plasma solute activity. If a typical 10-g larva continued to produce urine at the normal (tap water) rate after the osmotic entry of water had stopped, the solute concentration of body fluids would increase by about 2% in 2 h. The value would be higher for extracellular fluids. In mammals a 2% increase in osmotic pressure of extracellular fluid produces maximal antidiuresis (28). The possibility of external osmoreceptors in aquatic animals has not been ruled out.

The restoration of a normal GFR when the osmotic gradient is abruptly increased (200 mosM sucrose to tap water) is relatively slow. This may reflect the slow elimination of a hormone, such as AVT, or the presence of a relatively insensitive detection system, such as the renin-angiotensin system, which responds to hydration.

In addition to decreasing GFR a decreased osmotic load also increases the fractional reabsorption of water, while fractional reabsorption of solute decreases. Urinary concentrations of Na⁺, Cl⁻, and total solute were significantly elevated, a fact which has also been noted in chronically salt-loaded _A. tigrinum_ (12).

An increase in fractional reabsorption of water could reflect a selective increase in permeability of the renal tubules to water and/or an increased rate of active Na⁺ reabsorption with water following. Alternatively increased fractional reabsorption might result simply from a lower flow rate in the renal tubules and a resultant increased time for reabsorption. If changes in permeability or Na⁺ transport occur AVT could be involved. Mammalian posterior pituitary extracts increase fractional reabsorption of water in the distal tubules of _Necturus_ (7) and in larval _A. tigrinum_, hypophysectomy diminishes the increased tubular reabsorption of water associated with exposure to saline solutions (10). It is noteworthy that the fractional reabsorption of water in intact _A. tigrinum_ in isosmotic saline is much higher than we observed for _A. gracile_ in isosmotic sucrose (82% compared with 37%) (12). Salt loading may initiate different responses than simply decreasing the osmotic load on the animals.

The mechanism underlying the decreased fractional reabsorption of ions in salamanders in 200 mosM sucrose is not known. It could reflect a depression in the rate of active reabsorption of solute. We have observed a marginally significant decrease in Na⁺-K-activated ATPase in the microsomal fraction of kidney homogenates of _D. ensatus_ adapted to 200 mM sucrose (23). Alternatively it could reflect an increase in backflux of ions into the tubules. A sizable backflux of ions has been demonstrated in renal tubules of frogs (8) and _Necturus_ (5). Changes in the extent of the backflux have been associated with local changes in peritubular factors which affect ionic conductances along intercellular channels. In saline diuresis the backflux increases and there is a depression in the fractional reabsorption of Na⁺ (5).

Our data on renal function in intact, free-swimming, larval urodeles in tap water generally complement and support data obtained by micropuncture or other methods involving pithed or anesthetized animals and elaborate surgical procedures (11). For example, in pithed _Necturus_ creatinine clearance (GFR) is about 0.14 ml/10 g-h and the fractional reabsorption of water was 34% (11). Both values agree with our spring-fall data. Some differences are evident, particularly with respect to micropuncture data. Thus, Bott (4) found up to 50% of the filtered water in _Necturus_ is reabsorbed by the proximal tubules and in _Amblystoma_ the proximal tubules reabsorb 25–30% of filtered water and the distal segments another 20–95% (30). In micropuncture studies the U/P ratios for inulin or creatinine usually approach 2, whereas in intact ambystomats the value is usually less than 1.5 and may approach unity (12).

In larval _A. gracile_ (tap water) 92% of filtered Na⁺ is reabsorbed. A value of 95% has been reported for _Necturus_ using clearance methods (3) and 90% using micropuncture (30). Our values for fractional reabsorption of K⁺ and Cl⁻ are also very close to those obtained by micropuncture (4, 30).

The increase in the absolute reabsorption rate of Na⁺ and Cl⁻ as GFR increases probably reflects glomerulotubular balance, although glomerular intermittency may also be involved (see ref. 20). The mechanism of glomerulotubular balance is not known, but recent studies have focused on xonic and hydrostatic pressures of the peritubular capillary blood (15). Virtually all of these studies have been on mammals which normally maintain their GFR nearly constant. Consequently, to change the GFR it is necessary to clamp renal arteries, veins, or the ureters. In salamanders the GFR can be "set" by simply adjusting the osmotic concentration of the bath, which should make them well suited for studies of this type.

Freshwater animals must balance the ingress and egress of water and yet maintain their body fluids hypotonic to the external environment. This balance is maintained, in part, by adjusting the rate of urine flow to match the uptake...
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of water. This regulation is primarily achieved by altering glomerular filtration; however, increased reabsorption of urinary water occurs to some extent.

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