Osmoregulation and urea metabolism in the little skate *Raja erinacea*

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Goldstein, Leon, and Roy P. Forster. Osmoregulation and urea metabolism in the little skate *Raja erinacea*. Am. J. Physiol. 220(3): 742-746. 1971.—Little skates, *Raja erinacea*, were transferred gradually (4 days) from full to approximately half-strength seawater. Steady-state conditions (with respect to solute and water balance) were established by the end of the dilution program. The skates were maintained in the dilute environment for an additional 4 days and then gradually (4 days) returned to 100% seawater. Control groups were maintained in 100% seawater. Hematocrits were 20% lower and body weights were 18% higher in skates in dilute seawater than in controls. Plasma urea, chloride, and trimethylamine oxide concentrations were reduced 45, 30, and 27%, respectively, in dilute seawater. Urine flow and glomerular filtration rate were elevated six- and fourfold, respectively, in dilute seawater. The renal clearances of urea, chloride, and trimethylamine oxide increased 22, 6, and 13 times after environmental dilution. The percent filtered urea excreted by the kidneys increased sixfold in skates kept in 50% seawater. Total-body clearance of urea was similar in skates maintained in 100 and 50% seawater. Total urea excretion (production) was reduced in skates in dilute seawater. Thus, reduction in plasma urea concentration following environmental dilution was the result of increased renal clearance and decreased biosynthesis of the nitrogenous end product.

ammonia; trimethylamine oxide; kidney; sodium chloride

THE ABILITY of some clasmbranch species to live in both the sea and fresh or brackish water is well documented (14, 17). In order to exist in environments of such different salinities, these fish must possess mechanisms for maintaining solute-water balance. The major osmotic components of elasmobranch extracellular fluid are urea, sodium chloride, and trimethylamine oxide (TMAO) (3). In a study of the variations of urea and chloride concentrations in bloods of skates (*Raja eglanteria*) captured in seawater of different salinities or exposed to salinity changes under laboratory conditions, Price and Creaser (12) and Price (11) found that serum urea concentrations varied directly with the salinity of the medium. Serum chloride concentrations were less affected by the salinity changes. Goldstein, Oppelt, and Maren (7) found similar changes in the lemon shark (*Negaprion brevirostris*) and noted also that plasma TMAO concentrations were significantly lower in sharks maintained in dilute seawater than those kept in straight seawater.

The mechanisms by which the concentrations of solutes in the extracellular fluids of clasmbranchs are adjusted in environments of different salinities are unknown. Goldstein et al. (1) showed that the rate of biosynthesis of urea is not changed in lemon sharks adapted to dilute seawater but that the urea clearance from the body fluids of these fish is increased. Since urea is excreted by both the gills and kidneys of clasmbranchs (13), the increased clearance of urea from the body fluids of the fish in dilute seawater may be due to alterations in renal or branchial function or both. In the present study we investigated the roles of excretion and biosynthesis in the adaptation of blood urea concentration in the marine skate, *Raja erinacea*, to dilute seawater. Environmental dilution was found to markedly reduce the renal tubular reabsorption of urea. However, in contrast to the lemon shark, total-body clearance of urea was not significantly altered, and the rate of urea biosynthesis was reduced significantly by environmental dilution and then increased again by reconcentration of the environment.

MATERIALS AND METHODS

Little skates (*R. erinacea*) of mixed sex and weighing 700-1,300 g were captured by trawl line in Frenchman's Bay, Maine. They were maintained in a circular swimming pool (8 x 1.3 ft) supplied with both a seawater and freshwater hose. Dilution of the pool medium from 100 to 50% seawater was achieved by gradually (4-5 days) increasing the inflow of freshwater until it equalled the steady flow rate of seawater. Concentration of the pool back to 100% was done by slowly (4 days) decreasing the freshwater flow. The turnover rate for water in the pool was about 3 hr. Water chloride concentration was followed by assaying a pool sample with an Aminco-Cotlove (A. H. Thomas) automatic chloride titrator. The oxygen content of the pool, measured with an oxygen analyzer (Yellowstone Instruments model 51) equipped with a combination oxygen-temperature probe, was fairly constant (18-20%) at different salinities. The water temperature varied from 12-19°C, which is within the normal temperature range reported for *R. erinacea* (2).

Plasma concentration of urea, chloride, and trimethylamine oxide (TMAO) and the excretion rates of ammonia and urea were measured before dilution in 100% seawater (day 0). The skates were allowed to achieve a steady-state condition following pool dilution to 50% seawater before...
plasma and excretion values were again determined (days 6 and 7). Similar determinations were made 2 days after returning the pool to 100% seawater (day 14).

Blood samples were taken from a caudal vessel in heparinized syringes. These samples were centrifuged for 10 min in a clinical centrifuge, the hematocrits were recorded, and the plasma was decanted. The plasma proteins were precipitated with an equal volume of cold 10% (w/v) trichloroacetic acid. After remaining on ice for 10 min, the samples were centrifuged for 10 min and the supernatant solution was assayed for urea, chloride, and TMAO as described previously (7).

The rates of ammonia and urea excretion were measured by placing the individual skates in a plastic box containing 3 liters of water which was aerated. The box rested in a tub containing running seawater to maintain it at cool temperature (14–15°C). A sample was taken from the bath water after 3 hr and analyzed for urea and ammonia. Ammonia was assayed by the microdiffusion method using the color reagents described by Chaney and Marbach (4).

Renal clearance studies were done on two groups of male skates. One group (five male skates) was kept in 100% seawater in a live-car. The second group (four males) was kept in seawater which was gradually diluted to 50%, as described above, and were catheterized no sooner than 2 days after reaching 50% seawater. Urine was collected in a rubber balloon attached to an indwelling polyethylene cannula which was secured in the urogenital aperture of the middorsal cloacal wall by purse-string ligatures. Several additional ties to skin at the base of the tail anchored the catheter, and the fish was allowed to swim freely during urine collection periods. No sperm appeared in the urine, and the absence of inulin in alkaline (Marshall's) gland fluid at the end of clearance periods indicated that no retrograde movement of urine into the reproductive tract had occurred. Blood was collected from caudal vessels by ventral midline puncture of the tail with 24-gauge hypodermic needles.

Inulin clearance was used to measure glomerular filtration rates, and chemical determinations were made on cadmium filtrates of plasma and on diluted urine samples using the direct resorcinol method without alkali treatment (15). To achieve reasonably stable plasma inulin levels during the time of urine collections, intramuscular and subcutaneous injections were made into six to eight sites on the day before the clearance determinations were to be done. Plasma levels were then found to fall at a very slow rate. For example, a large skate weighing 5.2 kg was injected with 25 ml of a 3.3% inulin solution, and 24 hr later its plasma contained 70.5 mg/100 ml inulin. Then a clearance determination was begun, and at the end of a 6-hr urine collection period in seawater the plasma inulin had dropped to only 66.7 mg/100 ml. The urine flow was 0.25 ml/kg per hr, the midpoint U/P ratio was 2.27, and the glomerular filtration rate was 0.57 ml/kg per hr. Most of the experiments were run on smaller skates weighing approximately 1 kg, and injections of 3.0 ml of 3.3% inulin into these skates yielded plasma values of 25–35 mg/100 ml after 24 hr. In the smaller fish, single blood collections were made at the midpoint of the urine collection period to minimize blood loss.

Total-body clearance of urea-14C was measured by determining the rate of loss of injected urea-14C from the body fluids. The fish were injected with 1 μC urea-14C per kilogram in a caudal vessel. After allowing 3 days following injection for equilibration of labeled urea in the body fluid compartments, blood samples were taken periodically and analyzed for plasma urea-14C as described previously (7).

RESULTS

Figure 1 shows the time course for changing the salinity (Cl−) of the medium in which the skates (R. erinacea) were kept. Seawater (100%) was diluted to approximately 50% and then reconcentrated back to 100%. The skates did not appear to be adversely affected by the salinity changes. Body weights increased 11% during medium dilution (Table 1). A separate experiment showed that R. erinacea loses about 7% of its body weight per week when kept in 100% seawater without feeding. Thus, the skates kept in the diluted seawater had a net increase in body weight of 18%. Since these fish were not fed the weight gain must have

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**TABLE 1. Body weights, hematocrits, and plasma solute concentrations of R. erinacea following changes in environmental salinity**

<table>
<thead>
<tr>
<th>Seawater</th>
<th>Body Wt. (g)</th>
<th>% A Initial Body Wt</th>
<th>Hematocrit, %</th>
<th>Plasma Solute Concentrations, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Urea</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chloride</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TMAO</td>
</tr>
<tr>
<td>100%, Initial</td>
<td>1,058±48</td>
<td>15.5±0.7</td>
<td>396±11</td>
<td>48±3</td>
</tr>
<tr>
<td>30%</td>
<td>1,173±30</td>
<td>12.3±0.1</td>
<td>220±9</td>
<td>48±3</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.01</td>
<td></td>
<td>&lt;.01</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>100%, Final</td>
<td>902±36</td>
<td>14.1±1.2</td>
<td>443±19</td>
<td>40</td>
</tr>
<tr>
<td>P value*</td>
<td>&lt;.01</td>
<td></td>
<td>&lt;.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers of fish per group are shown in parentheses. * P values refer to groups in 50 and 100% (final) seawater compared to group in 100% seawater (initial).
been due to increased water uptake. This conclusion is consistent with the observation that hematocrits decreased about 20% during the dilution program (Table 1).

As shown in Fig. 1, the concentration of urea, chloride, and TMAO in the plasma fell to new steady-state levels by the end of the 1-week dilution period. Urea concentration decreased approximately 45%, chloride, 30%, and TMAO, 27%. Part of these changes may have been due to hemodilution as judged by the lowered hematocrits. Since urea, sodium chloride, and TMAO account for most of the osmotically active solutes of elasmobranch plasma (3), the total of these three solutes should approximate the plasma osmolality. The estimated plasma osmolality (urea + chloride X 2 + TMAO) of R. erinacea in 100% seawater is slightly hypertonic to the medium (1,010 vs. 950 mOsm), whereas the estimated plasma osmolality of skates in 50% seawater is markedly hypertonic to the medium (660 vs. 480 mOsm). During the 1-week period in which the salinity of the pool was returned gradually from 50 to 100%, seawater plasma urea and chloride concentrations increased to levels similar to those at the start of the experiment while plasma TMAO concentration remained somewhat low. Hematocrits rose above initial levels. Body weights decreased markedly following return of the skates to 100% seawater, probably the result of both starvation and loss of excess water that accumulated during the dilution part of the experiment.

Renal function was assessed in skates maintained in 50% seawater and compared with that in skates kept in 100% seawater. As shown in Table 2, urine flow was 6 times greater and glomerular filtration rate was 4 times higher in skates maintained in 50% seawater as compared to those in 100% seawater. The renal clearances of urea, chloride, and TMAO were 22, 6, and 13 times greater, respectively, in skates in 50% seawater as compared to those in 100% seawater. As shown in Table 2, the percent of filtered urea that was excreted by the renal tubules increased nearly sixfold in skates maintained in dilute seawater. The percentages of filtered chloride and TMAO excreted in the urine were not significantly changed in skates maintained in 50% seawater.

Urea clearances from the body fluids of skates maintained in 50% or 100% seawater were determined by measuring the rates of total urea loss from the fish and the plasma concentrations of urea which were assumed to be in equilibrium with extravascular body fluids. Clearance was then calculated using the equation $C_\text{urea} = \frac{R_\text{urea}}{P_\text{urea}}$, where $C_\text{urea}$ is the total-body clearance, $R_\text{urea}$ is the rate of total urea excretion, and $P_\text{urea}$ is urea concentration per milliliter of plasma. As shown in Table 3, urea clearances were similar in skates maintained in diluted and undiluted seawater (0.72 ml/kg per hr vs. 0.83 ml/kg per hr). This finding was not unexpected in view of the marked elevation in renal urea clearance in skates kept in diluted seawater. We therefore sought to confirm these results by another method of assessing the clearance of urea from body fluids. Total-body clearance of urea may be calculated from the rate of loss of injected urea-14C from the body fluids using the equation $C_\text{urea} = \frac{R_\text{urea}}{P_\text{urea}}$, where $R_\text{urea}$ is the rate of loss of urea-14C from plasma and the fraction of injected urea-14C remaining in the body fluids at time $t$. The urea space was assumed to be equal to total-body water—62% of the body weight (16). It was also assumed that the rate of loss of urea-14C from plasma, after an equilibration period of 2-3 days, was equal to the rate of loss of this compound from total-body fluids. As shown in Fig. 2, the rate of loss of urea-14C from plasma was similar in skates maintained in 50% seawater (1.95%/day) and 100% seawater (2.0%/day). The calculated total body urea clearances for fish in 100 and 50% seawater were 0.76 and 0.44 ml/kg per hr, respectively, which compare favorably with the values of 0.83 and 0.72 ml/kg per hr obtained with the direct method (see above).

The rate of total urea excretion was significantly lower in the skates in diluted seawater than that in skates in undiluted seawater (Table 3). Since the rate of urea excretion was increased when the salinity of the medium was raised from 30 to 100% seawater, the fall in rate of urea excretion during the dilution program was probably not due to starvation but rather more related to the salinity change. In addition separate experiments (not shown) showed that maintaining skates in 100% seawater for 1 week without feeding had no significant effect on the rate of urea excretion. The rate of ammonia excretion was not altered by changes in the salinity of the environment (Table 3).

**DISCUSSION**

The results obtained in this study show that the skate, R. erinacea, can adapt to environmental dilution and re-

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**TABLE 2. Effect of environmental dilution on renal function in skates, R. erinacea**

<table>
<thead>
<tr>
<th>Seawater</th>
<th>Urine Flow, ml/kg per hr</th>
<th>GFR, ml/kg per hr</th>
<th>Renal Clearance, ml/kg per hr</th>
<th>Excreted/Filtered, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>0.19 ± 0.02</td>
<td>0.62 ± 0.18</td>
<td>Urea: 0.03 ± 0.01</td>
<td>6 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloride: 0.12 ± 0.02</td>
<td>27 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TMAO: 0.025 ± 0.01</td>
<td>6 ± 1.5</td>
</tr>
<tr>
<td>50%</td>
<td>1.3 ± 0.1</td>
<td>2.6 ± 0.3</td>
<td>Urea: 0.2 ± 0.1</td>
<td>26 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloride: 0.11 ± 0.01</td>
<td>9 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TMAO: 0.32 ± 0.02</td>
<td>21 ± 5.3</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.02 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Number of animals per group shown in parentheses.

**TABLE 3. Urea and ammonia excretion and total-body urea clearance following changes in environmental salinity**

<table>
<thead>
<tr>
<th>Seawater</th>
<th>Urea Excretion, µmol/kg per hr</th>
<th>Ammonia Excretion, µmol/kg per hr</th>
<th>Total-Body Urea Clearance, ml/kg per hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%, Initial</td>
<td>239 ± 42 (6) &lt; P &lt; 0.05</td>
<td>111 ± 15 (6) &lt; P &lt; 0.4</td>
<td>0.03 ± 0.15 (4) &lt; P &lt; 0.7</td>
</tr>
<tr>
<td>50%</td>
<td>126 ± 16 (6) &lt; P &lt; 0.05</td>
<td>126 ± 8 (6) &lt; P &lt; 0.2</td>
<td>0.72 ± 0.20 (4)</td>
</tr>
<tr>
<td>100%, Final</td>
<td>214 ± 29 (6)</td>
<td>104 ± 4 (6)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. Numbers of fish per group shown in parentheses. * P values refer to group in 50% seawater compared to group in 100% seawater (initial). † P values refer to group in 100% seawater (final) compared to group in 50% seawater.
OSMOREGULATION IN SKATES

concentration. R. erinaceus is strictly a marine species (2). However, other skates, e.g. R. eglanterin, are found in both brackish and full-strength seawater (11). Although R. erinaceus can survive in 50% seawater, it has difficulty maintaining solute-water balance. The concentrations of the major solutes of the extracellular fluids (urea, TMAO, and NaCl) are reduced in fish in the dilute environment, but the osmotic pressure of the body fluids remains significantly higher than the surrounding medium. The increased osmotic gradient between the fish and the environment led to a net uptake of water as evidenced by reduced hematocrit and increased body weight. This is in marked contrast to the situation in the lemon shark, N. brevirostris (7), in which no significant change in hematocrit was found following adaptation to 50% seawater. The ability of the lemon shark to maintain solute-water balance in a dilute environment more successfully than the little skate is not surprising, since the former normally inhabits brackish water (1).

In their studies on the adaptation of the lemon shark to environmental dilution, Goldstein, Oppelt, and Marcus (7) found that the reduction of urea concentration in the body fluids which accompanied this procedure was brought about mainly by increased excretion of the nitrogenous end product. The site and mechanism of the increased excretion were not studied. Urea is excreted by the gills and kidneys of elasmobranchs, the major portion being excreted by the gills (13). Smith (13) reported that urine flow in the elasmobranch, Pristis microdon, in freshwater was 30-100 times that observed in marine elasmobranchs. In the present study, urine flow was increased sixfold in skates maintained in 50% seawater. The large increases in urine flow undoubtedly contributed to the increased excretion of urea as well as chloride and TMAO. Two other factors, however, played a role in the elevation of urea excretion. First, glomerular filtration rate was elevated fourfold in skates maintained in 50% seawater. Second, the reabsorptive transport of urea across the renal tubules was markedly inhibited by environmental dilution. Skates maintained in 100% seawater excreted 6% of urea filtered at the glomerulus, whereas those in 50% seawater excreted 34% of the filtered urea. The effect was specific for urea in that the percent filtered chloride and TMAO that were excreted were only marginally affected by environmental dilution. The processes operating to reduce renal tubular urea reabsorption following reduction in environmental salinity are not known. Several factors which might have led to altered urea reabsorptive capacity in the nephron are: 1) increase in glomerular filtration rate, 2) inhibition of the release of a renotropin hormone, 3) changes in the physical reabsorptive forces operating across the renal tubular cells. All three of these factors have been invoked to explain the naturesis that occurs following expansion of the extracellular fluid in mammals (5). There is no evidence available to suggest which, if any, of these factors may play a role in the regulation of urea reabsorption in the elasmobranch kidney.

Since approximately 20% of the urea excreted by elasmobranchs is eliminated by the kidneys, the 20-fold increase in renal urea clearance in skates adapted to 50% seawater would be expected to lead to significant elevation in total-body urea clearance. In fact, however, total-body urea clearance, measured by two independent methods (Table 3 and Fig. 2), showed that this clearance was similar in fish in 50 and 100% seawater. One must conclude, therefore, that a decrease in branchial urea clearance accompanied the increase in renal urea clearance in skates maintained in 50% seawater. Potts et al. (10) reported that water permeability of the gills of euryhaline teleost, Tilapia mossambica, was significantly less in seawater than in freshwater, and Motais et al. (9) found that both diffusion- and osmotic water permeability of the gills of the euryhaline teleost, Platichthys flesus and Anguilla anguilla, were less in seawater than in freshwater. However, Evans (6) found no such difference in similar studies on the intertidal teleost, Xiphister alutatus, and Goldstein (unpublished data) found that the exchange of H2O across the gills of the lemon shark was unchanged by environmental dilution.

The constancy of total-body urea clearance in skates maintained in 50 and 100% seawater contrasts with the situation in the lemon shark where environmental dilution leads to significant increases in total-body urea clearance. Furthermore, urea production was not altered by environmental dilution in lemon sharks, but was reduced nearly twofold in skates by the same procedure. The inhibition of urea production in the latter species was partly responsible for the observed reduction of blood urea concentration in skates adapted to 50% seawater. The mechanism effecting a reduction of urea biosynthesis in this situation is unknown. Ammonia production was not affected by environmental dilution; thus, one cannot attribute the decrease in urea biosynthesis to a general reduction in nitrogen catabolism. Urea biosynthesis returned toward predilution levels when the skates were returned to 100% seawater, indicating that the effects of environmental dilution were reversible. The effects of salinity changes on urea biosynthesis in skates resemble those observed in Xenopus (8). In Xenopus an increase in en-

FIG. 2. Plasma disappearance of urea-14C. Skates were injected with approximately 1 µC/kg urea-14C (4.6 µC/mole) intravenously 2-3 days before day 1. Seven fish were divided into 2 groups. One group (4 fish) was maintained in 100% seawater (x---x), the other (3 fish) in 50% seawater (- -). For latter group, seawater was gradually diluted to half-strength from days 1 to 5. Blood samples were taken on days shown and plasma was assayed for urea-14C. Lines fitted to points on graph using regression equation. Points from days 1 to 7 were used to fit line for undiluted group. Points from days 5 to 9 only were used to fit line for diluted group.
vironmental salinity leads to augmentation in urea biosynthesis with little reduction in ammonia excretion (8). Thus, in both Xenopus and Raja changes in environmental salinity alter urea biosynthesis with little or no effect on ammonia production. Whether the mechanism(s) responsible for bringing about the changes in urea metabolism are similar in the two species remains to be investigated.

REFERENCES