Osmotic regulation and urea metabolism in the lemon shark *Negaprion brevirostris*

**Leon Goldstein, W. Walter Oppelt, and Thomas H. Maren** (With the Technical Assistance of Deborah Funkhouser)

Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115; Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida 32601; and The Lerner Marine Laboratory, Bimini, Bahamas

Lemon sharks *Negaprion brevirostris* were transferred gradually (within 1 wk) 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 sharks were then maintained in the dilute environment for an additional week. A control group was maintained in seawater. Hematocrits of the fish in dilute seawater were similar to those in the control group, suggesting that the sharks in the dilute environment maintained water balance. Plasma urea, trimethylamine oxide, and chloride concentrations were reduced 55, 60, and 20%, respectively, in the sharks in diluted seawater compared to the control group. Urea clearance from the body fluids was approximately three times greater in the group in diluted seawater compared to the controls. The rates of total urea excretion in the steady state (equal to urea production) were similar in the two groups. These results suggest that lemon sharks adapt to environmental dilution by reducing the concentrations of the major extracellular solutes—urea, NaCl, and trimethylamine oxide. The reduction in urea concentration is due mainly to an increase in urea clearance and not a change in biosynthesis under these conditions.

Although most elasmobranchs live in the sea, numerous species of sharks, rays, and skates enter and live in brackish and freshwater for various periods of time (12, 14). In order to migrate from a marine to a freshwater environment and back, these fish must possess mechanisms for maintaining solute-water balance in the different salinities. Smith (11) observed that the bloods of elasmobranchs found in freshwater contained significantly less urea and chloride than the bloods of marine elasmobranchs. Thorson and co-workers recently confirmed Smith's findings in studies on the body fluid composition of the freshwater ray, *Potamotrygon* sp. (15) and the euryhaline bullshark, *Carcharias* *leucas* (14) captured in freshwater. In a study of the variations of salt and urea concentrations in blood of the same species in different salinities, Price (7) found that serum urea concentrations in the skate (Raja *eleganteria*) varied directly with the salinity in which the fish were found, but that salt concentrations were unaffected by the concentration of the environment. Price and Creaser (8) carried out similar studies in skates exposed to salinity changes under laboratory conditions and showed that serum urea and, to some extent salt concentrations, decreased as the salinity of the environment was lowered. The effect of salinity changes on the body fluid concentration of trimethylamine oxide, an organic base thought to have osmoregulatory function (3), has not been examined previously in elasmobranchs.

The mechanisms which operate to regulate the concentrations of urea and other solutes in body fluids of elasmobranchs migrating from an environment of one salinity to another are unknown. Studies on euryhaline amphibia (5, 9) showed that the urea concentrations in the body fluids of these animals were changed in different salinities. These changes were brought about by altering the rates of excretion and biosynthesis of the compound; in going from freshwater to brackish water or saline, urea concentration in the body fluids was elevated by reduction in the rate of excretion and increase in the rate of biosynthesis. The purpose of the present investigation is to determine whether or not similar changes in the rates of urea excretion and biosynthesis operate to regulate the concentration of urea in the body fluids of the euryhaline elasmobranchs when environmental salinity is changed under laboratory conditions. The elasmobranch chosen for this study was the lemon shark *Negaprion brevirostris* which is known to enter freshwater (1). Additional data were obtained on changes in plasma concentrations of...
chloride and trimethylamine oxide under these conditions.

METHODS

Animals and treatments. Lemon sharks (Negaprion brevirostris) were captured by handline offshore in Bimini, Bahamas. The sharks, of mixed sex and weighing 2–3 kg, were divided into two groups and maintained without feeding. One group was maintained in a pen placed in a seawater lagoon. The other group was maintained in aquaria supplied with a mixture of seawater and brackish water. Water in the aquaria was diluted by maintaining the flow of seawater into the tank as constant as possible and gradually increasing the flow of brackish water until the desired salinity was achieved. Variation in salinity of both seawater and brackish water were common, and the relative flow rates of seawater to brackish water had to be adjusted frequently to maintain the desired salinity. Salinity was monitored using a conductivity meter (Barnstead model PM-70CB) with a 10-K cell (Industrial Instruments model VH10). Water chloride concentration was measured by titration with phenylmercuric nitrate (13). Water temperature, pH, and oxygen content were determined daily. Oxygen content was measured with an oxygen analyzer (Yellowstone Instruments model 31) equipped with a combination oxygen-temperature probe. Water pH was measured with a portable pH meter (Beckman model N-2).

After the fish were in a steady state, as judged by stability of plasma urea concentrations, 2–3 µc of urea-14C (New England Nuclear, 16 µc/mnmole), was injected in a caudal blood vessel. The day of injection is referred to as day 0 of the experiment. Blood samples (approximately 3 ml) were removed from a caudal vessel on day 1 and every other day thereafter for 1 week. The samples were spun in graduated centrifuge tubes for 10 min in a clinical centrifuge, the hematocrits recorded, and the plasmas separated.

Plasma analysis. Plasma proteins were precipitated by mixing equal volumes of plasma and cold 10% trichloroacetic acid and allowing the mixture to stand in the cold for 10 min. The samples were spun in a clinical centrifuge for 10 min. The clear supernatant solution was decanted, for 10 min. The samples were spun in a clinical centrifuge, the hematocrits recorded, and the plasmas separated.

Calculation of total urea excretion (production). Under steady-state conditions urea production is equal to urea excretion. Thus, urea excretion was measured after plasma urea levels had stabilized. Since urea excretion occurs via the gills and the kidneys and could not be measured directly, an indirect method was used. This method consisted of injecting a tracer amount of urea-14C into the circulation, allowing time for equilibration of the radioactive urea in the various body fluids and then determining the rate of loss of urea-14C from the body fluids. Rate of total urea excretion was then calculated from the equation: 

\[ r_{\text{urea}} = k \cdot P_{\text{urea}} \cdot S_{\text{urea}}, \]

where

\[ r_{\text{urea}} = \text{rate of total urea excretion (µmoles/kg body wt per day)}; k = \text{rate of loss of urea from plasma (%/day)}; P_{\text{urea}} = \text{plasma total urea concentration (µmoles/ml)}; S_{\text{urea}} = \text{urea space or volume of body fluids in which urea is distributed (ml/kg body wt). Urea space was assumed to be 65% of total body weight, the value reported by Murdaugh, Robin, and Hearn (6) for the urea space in the elasmobranch Squalus acanthias. The rate of loss of total urea from plasma was assumed to be equal to that of urea-14C loss.}

RESULTS

Figure 1 shows the time course of reducing the salinity (Cl–) of the medium in which the lemon sharks (Negaprion brevirostris) were kept from full to approximately 50% salinity.
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half-strength seawater. The sharks tolerated the dilution program well and at no time did they appear to be distressed by the treatment. The group in diluted seawater was then maintained at approximately half-strength seawater during the course of the experiment (Fig. 2A). A control group was maintained in full-strength seawater and handled in a manner similar to that of the fish in the diluted environment. With both groups we compared three environmental factors which might have had an effect on the metabolism of the fish and found no significant differences between the two environments. These factors were water temperature, oxygen content, and pH (Table 1).

Blood samples were taken from fish maintained in the diluted and undiluted environments about every other day and analyzed for hematocrits, chloride, urea, and trimethylamine oxide concentrations. As shown in Fig. 2, B, C, and D, the concentrations of the solutes had reached new steady-state levels by the end of the 1 week dilution period (day 0) and remained relatively stable for the next week. Hematocrits were similar in sharks maintained in diluted and undiluted environments (Fig. 3) indicating that the vascular volume was not altered by dilution which supports the view that the fish were maintaining water balance in the dilute environment. Comparing sharks maintained in dilute environment with those maintained in seawater (Fig. 2, B, C, and D, and Table 2) dilution caused the following reductions in plasma solute concentrations: urea, 55%; chloride, 20%; and trimethylamine oxide, 60%. Since urea, NaCl, and trimethylamine oxide account for most of the osmotically active solutes of elasmobranch plasma (2), the total of these three solutes should approximate the plasma osmolarity. As shown in Table 2, the total of these three solutes in the plasma of the fish in the diluted seawater was approximately 60% of that of those kept in straight seawater. However, in contrast to the latter group of fish, which were approximately isosmotic with their environment, the former group remained definitely hypertonic to the diluted seawater (Table 2).

Urea excretion was measured indirectly by determining the rate of loss of urea-14C from the sharks. The fish were injected with a tracer dose of radioactive urea on day 0 of the experiment and blood samples were taken 24 hr after injection and usually every other day thereafter, for 1 week. As shown in Fig. 4, the rate of loss of urea-14C from the plasma occurred at a linear exponential rate after the 3rd day. The rate of loss of urea-14C from the plasma (%/day) from days 3 to 7 was calculated for individual fish and the means are shown in Table 3. The rate of loss of urea from sharks in the undiluted environment was 1.7%/day, whereas the rate of loss from fish in the diluted environment was 4.8%/day. Thus, under steady-state conditions the clearance of urea-14C from the body fluids was 2.8 times greater in the fish in the diluted seawater than in fish in undiluted seawater.

Under steady-state conditions the rate of production

TABLE 1. Temperature, oxygen content, and pH of diluted and undiluted seawater

<table>
<thead>
<tr>
<th>Seawater</th>
<th>Temperature, °C</th>
<th>Oxygen Content, vol %</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted</td>
<td>26 ± 0.2</td>
<td>16 ± 0.7</td>
<td>7.55</td>
</tr>
<tr>
<td>Undiluted</td>
<td>24 ± 0.9</td>
<td>18 ± 0.7</td>
<td>7.95</td>
</tr>
</tbody>
</table>

Values are averages of seven daily measurements for temperature, five for oxygen content, and two for pH. SE is shown for temperature and oxygen content.

FIG. 3. Hematocrits of lemon sharks maintained in undiluted and diluted seawater.
of urea is approximately equal to the rate of excretion. Thus, one can determine the rate of production of urea by calculating the rates of total urea excretion. As shown in Table 3, the rate of total urea excretion in the undiluted group of lemon sharks was 4,580 pmoles/kg per day, which is within the range (2,400–7,200 pmoles/kg per day) found in the dogfish *Squalus acanthias* (J. W. Boylan, personal communication). The rate of urea production was either unchanged or slightly elevated (.1 > P > .05) in sharks maintained in the diluted environment.

### TABLE 2. Plasma solute concentrations in lemon sharks maintained in diluted and undiluted seawater

<table>
<thead>
<tr>
<th>Group</th>
<th>Undiluted</th>
<th>Diluted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma chloride, mm X 2</td>
<td>620 ± 10</td>
<td>504 ± 4</td>
</tr>
<tr>
<td>Plasma urea, mm</td>
<td>421 ± 2</td>
<td>191 ± 4</td>
</tr>
<tr>
<td>Plasma TMAO, mm</td>
<td>76 ± 4</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Total, mm</td>
<td>1,117</td>
<td>726</td>
</tr>
<tr>
<td>External medium, mosmols</td>
<td>1,130</td>
<td>530</td>
</tr>
</tbody>
</table>

Values are means ± SE of plasma samples taken from six fish per group maintained for 7 days in diluted or undiluted seawater.

![Graph](image)

**FIG. 4.** Plasma disappearance of urea 14C. Lemon sharks were injected with approximately 1 μg/kg urea-14C (16 μC/m mole) intravenously on day 0. Blood samples were taken on days shown and plasmas assayed for urea-14C.

### TABLE 3. Urea-14C and total urea excretion in lemon sharks kept in diluted and undiluted seawater

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea-14C Excretion, %/day</th>
<th>Plasmas Urea, μmoles/ml</th>
<th>Total Urea Excretion, μmoles/kg X day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>1.7 ± 0.2</td>
<td>420 ± 5</td>
<td>4,360 ± 620</td>
</tr>
<tr>
<td>Diluted</td>
<td>4.8 ± 0.2</td>
<td>189 ± 2</td>
<td>5,950 ± 320</td>
</tr>
</tbody>
</table>

Values are means ± SE of five to six fish per group. Calculated from the equation R_urea = k · P_urea · S_urea, where R_urea = rate of total urea excretion (μmoles/kg body wt per day); k = rate of loss of urea-14C from the plasma (%/day); P_urea = plasma total urea concentration (μmoles/ml); S_urea = urea space (ml/kg). Rate of loss of total urea from plasma was assumed to be equal to that of urea-14C loss.

### DISCUSSION

Elasmobranchs are found in all the oceans, most seas, and many rivers. This highly successful colonization of the various waters of the world is dependent in large part on the ability of these animals to maintain solute and water balance in the face of a wide variety of environmental conditions. The major osmoregulatory mechanism that allows elasmobranchs to maintain osmotic equilibrium or superiority to a marine environment is the accumulation of urea and trimethylamine oxide in the body fluids of these organisms. Previous studies and those presented here show that the levels of both urea and TMAO, and to a lesser degree NaCl, are affected in elasmobranchs when environmental salinity changes. The mechanisms operating to regulate the concentration of urea and trimethylamine oxide in the body fluids of elasmobranchs are not well known. Urea is produced in the liver of elasmobranchs mainly via the ornithine-urea cycle (10). The origin of trimethylamine oxide is unknown (4). The high concentration of these organic solutes is not due to unusually high rates of production but rather to the existence of separate active transport systems in the kidneys of elasmobranchs that retain over 95% of the urea and TMAO filtered at the glomerulus (3).

On placing lemon sharks in diluted seawater, we observed reductions of 55% in plasma urea concentrations and 60% in plasma TMAO concentrations. The simplest explanation for these results is that the increased influx of water across the body surfaces, e.g., gills, in the dilute environment resulted in a large increment in urine flow and subsequent increased losses of urea and TMAO in the urine. Smith (11) found that urine flow in the freshwater elasmobranch *Pristis microdon* was 50–100 times that observed in marine elasmobranchs. Since most of the urea excreted by elasmobranchs (11) leaves via the gills, this increase in urine flow would be expected to have a relatively small effect on the rate of total urea clearance. In the present study we observed an approximate three-fold increase in total urea clearance in sharks maintained in diluted seawater as compared to those kept in an undiluted environment. This observation is consistent with the view that the increased loss of urea in diluted sharks was the result of a physiological diuresis that these animals experience in a dilute environment. On the other hand it is possible that gill permeability and the active transport of urea by the renal tubules were both effected by dilution and that these factors also contributed to the increased loss of urea under this condition.

In view of the recent report (5) that the rate of urea biosynthesis was increased during osmotic stress in the amphibian *Xenopus laevis*, we examined the possibility that the opposite response might occur in sharks upon dilution of the environment. However, the results obtained with the technique used in the present study indicate that under steady-state conditions there is no significant decrease in the rate of urea production in lemon sharks kept in half-strength seawater. It is unfortunate...
that this technique depends on the existence of steady-state conditions and, therefore, urea biosynthesis could not have been measured during the transitory phase of the dilution program. Also, it was impractical to study the sharks during prolonged periods (weeks or months) of dilution which might have lead to decreases in the rates of urea biosynthesis. If these decreases do take place then one would expect to find that the proportion of nitrogen excreted as urea compared to other nitrogenous end products, e.g., ammonia, would decrease during prolonged dilution, unless the total metabolic rate decreased. However, Smith (11) found that Pristis microdon which had been living in freshwater for prolonged periods was still ‘ureotelic’; that is they excreted approximately 70-80% of the total urea + NH$_4$-N eliminated as urea. Although these data cannot be taken as proof of a lack of change in urea biosynthesis in elasmobranchs migrating into freshwater they do argue against a drastic alteration in the urea biosynthetic machinery. If the results obtained in the lemon shark are typical of elasmobranchs in general, then it appears that the adaptations of body fluid urea concentrations to variations in environmental salinity are brought about by changes in excretion and not biosynthesis of the compound.

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