## Excretion of Hypertonic Urine by a Teleost

Abstract. *During the course of adaptation to sea water,* Fundulus kansae *excretes urine that is hypertonic to the blood, but hypotonic to sea water. During this period the osmotic pressure of the serum is greater than that found in animals adapted to fresh water or sea water. The urine collected from fish adapted to sea water is usually isotonic to the blood.*

It has long been a postulate of comparative animal physiology that teleosts are not able to excrete urine that is hypertonic to the blood. This generality can be credited to Smith (*1*) although earlier comparisons of the osmotic pressure of the urine and serum of the teleost had been made (*2*). Moreover, no exceptions to this idea have been found (*3*). We wish to report, that under certain conditions, a euryhaline teleost, *Fundulus kansae,* can excrete hypertonic urine. The ability of this species to tolerate high salt concentrations has been reported (*4*).

The fish were collected from a salt spring, "Boonslick," located in Howard County, Missouri. The animals were placed in large concrete holding tanks and provided with running tap water and adequate aeration. The water supply at the university is from deep wells and is not chlorinated. They were held for 2 months before use. They were fed daily and thrived in captivity. Only prespawning females weighing between 1.1 g and 1.9 g were used. Experiments were carried out at 19° ± 0.5°C.

Sea water was made up to a salinity of 34.5 parts per thousand by reconstituting evaporated sea water (Rila Marine Mix) with deionized water. This solution contained 440 meq of $Na^+$ per liter, 21 meq of $Ca^{++}$ per liter, and 14 meq of $K^+$ per liter; it was equivalent, osmotically, to a 0.53*M* NaCl solution.

Urine was collected by inserting a small glass catheter made by drawing microhematocrit tubing into the urogenital papilla and stitching it firmly in place. Polyethylene tubing (Clay-Adams, P.E. 190) was attached to the catheter. The catheter and tubing were marked in 5-$\mu$l divisions with a nonwettable ink, and the rate of urine production was measured directly.

After the catheters were fixed, the fish were placed in individual glass tubes that were drawn to a diameter of 5 mm at one end. This prevented the fish from swimming through the openings but did not restrict the flow of water through the chambers. The diameter of the chambers was large enough so that fin movement would not be restricted, but small enough so that the fish could not turn around. The back of each chamber was covered with a plastic screen which prevented the fish from backing out. The chambers were held in a plexiglass aquarium containing 5 liters of water. The aquarium water was kept saturated with oxygen.

Urine samples were withdrawn by carefully threading a length of small polyethylene tubing (Clay-Adams, P.E. 10) inside the collection tubing and applying gentle suction.

Samples were withdrawn in order to measure the urine osmotic pressure (Mechrolab vapor pressure osmometer), and sodium, calcium, and potassium. The ions were estimated with a Coleman flame photometer.

*Fundulus kansae* behave well under these experimental conditions. We have maintained experiments for as long as 120 hours without visible damage to the fish.

Animals treated in the following ways were studied. (i) Animals adapted to fresh water were catheterized and returned to fresh water. At the end of 24 hours the accumulated urine was removed and discarded. The urine produced during the following 6-hour period was collected for analysis. (ii) Fresh-water adapted animals were catheterized and placed directly in sea water. At the end of 3 days the accumulated urine was removed and discarded. The urine produced during the following 24 hours was collected for analysis. (iii) Fish adapted to sea water for 20 days were catheterized and returned to sea water. The urine produced during the next 2 days was discarded, and the urine excreted during the 3rd day was taken for analysis. The initial urine produced in each case was rejected since studies on the rates of glomerular filtration of animals adapted to both tap water and sea water have shown that these animals are always diuretic for a variable period after cannulation (*5*). In every case the animals were killed, and blood was collected (*6*) as soon as the urine samples were taken.

The volume of the urine and the osmotic pressure of the urine and serum of individual animals are given in Table 1. Table 2 shows concentrations of $Na^+$, $K^+$, and $Ca^{++}$ in the urine and serum from the same animals. The data in Table 1 show that *F. kansae* held in tap water produces a copious dilute urine. After transfer to sea water, urine production dropped markedly, and the urine excreted on the 3rd day after the transfer is definitely hypertonic to the serum. Data not reported here show that this condition is maintained for at least 10 days after transfer into sea water; by 20 days, however, the urine is in most cases isotonic or slightly hypotonic to the blood. Thus, while *F. kansae* is capable of excreting hypertonic urine, it appears that this fish does not continue to do so.

It might be argued that the data in Table 1 suggest that the fish held in sea water for 20 days were somewhat diuretic, especially since Forster and Berglund (*7*) have reported that the urine of a marine teleost, known to be diuretic, was isotonic to the blood. Such a comparison may not be valid, however, because Forster and Berglund's studies were carried out on an aglomerular teleost where, presumably, the mechanisms required for urine formation would be somewhat different from those required here. Adapted to

Table 1. Urine flow and urine and serum osmotic pressure as millimoles of NaCl per liter in *Fundulus kansae* held in fresh water and sea water.

| Daily urine flow (ml/kg) | Osmotic pressure (mmole/lit.) | |
|---|---|---|
| | Urine | Serum |
| | *Tap water* | |
| 298 | 8.3 | 176 |
| 75 | 16.3 | 171 |
| 212 | 15.7 | 181 |
| 170 | 14.1 | 183 |
| 245 | 13.8 | 187 |
| 200 | 16.4 | 188 |
| 200 ± 38 | 14.1 ± 0.7 | 181 ± 3 |
| | *Sea water for 3 days* | |
| 7 | 272 | 205 |
| 4 | 491 | 227 |
| 14 | 322 | 269 |
| 10 | 383 | 328 |
| 8 | 295 | 233 |
| 6 | 413 | 224 |
| 12 | 355 | 207 |
| 9 ± 1 | 362 ± 28 | 242 ± 16 |
| | *Sea water for 20 days* | |
| 16 | 162 | 186 |
| 31 | 183 | 190 |
| 22 | 191 | 191 |
| 23 | 185 | 193 |
| 26 | 184 | 190 |
| 9 | 198 | 190 |
| 21 ± 3 | 184 ± 5 | 190 ± 1 |

Table 2. Urine and serum sodium, calcium, and potassium in milliequivalents per liter in *Fundulus kansae* held in fresh water and in sea water (S.W.) (mean ±S.E.).

| Treatment | Specimen No. | Urine | Serum |
|---|---|---|---|
| | | *Sodium* | |
| Tap water | 6 | 12.8 ± 0.7 | 160 ±2 |
| 3 days in S.W. | 5 | 261 ±65 | 231 ±2 |
| 20 days in S.W. | 6 | 140 ±16 | 157 ±1 |
| | | *Calcium* | |
| Tap water | 6 | 0.4 ± 0.1 | 4.9 ±0.2 |
| 3 days in S.W. | 5 | 12.8 ± 2.1 | 9.2 ±0.7 |
| 20 days in S.W. | 6 | 19.9 ± 2.4 | 6.0 ±0.4 |
| | | *Potassium* | |
| Tap water | 6 | 0.17 ± 0.01 | 3.88 ±0.31 |
| 3 days in S.W. | 5 | 6.4 ± 3.2 | 2.00 ±0.42 |
| 20 days in S.W. | 6 | 2.0 ± 0.1 | 1.20 ±0.10 |

sea water, *F. kansae* does have functional glomeruli (*5*). The levels of urine production reported in Table 1 for adapted fish has been seen repeatedly, and there seems to be no reason to suppose that the fish are diuretic. Forster and Berglund found that the plasma osmotic pressure rose slowly in the diuretic fish and also noted marked shifts in the electrolyte content of the diuretic urine. No such shifts were seen here, and the osmotic pressure of the serum taken from the adapted fish was close to that found in fish held in tap water.

Finally, it should be pointed out that in no case did the osmotic pressure of the urine exceed that of sea water. While the osmotic pressure of this fluid is largely caused by sodium salts (Table 2), the sodium concentration of the hypertonic urine was, on the average, 180 meq/lit. less than that of the sea water. Thus, the kidney would never be able to handle the salt load imposed on the animal as a result of drinking sea water. Considerable salt must still be eliminated by another route, presumably the gills.

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**References and Notes**

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## Freezing and Viability of Tetrahymena pyriformis in Dimethylsulfoxide

Abstract. *Ciliated protozoa may be preserved at very low temperatures.* Tetrahymena pyriformis, *suspended in 10 percent dimethylsulfoxide, survived when frozen in two steps, first to −20°C, then transferring immediately to a temperature of −196°C. Motility and ability to reproduce were recovered after freezing by this method and subsequent storage for 3 months in a liquid nitrogen refrigerator.*

Some amoebae have been frozen in the presence of glycerol (*1*) and have remained viable. Of the flagellated protozoa some of the trichomonads (*2, 3*) and trypanosomes (*4, 5*) have been subjected to freezing and thawing and have remained viable, but no reports of successful attempts to freeze and store cultures of ciliated protozoa at very low temperatures have come to our attention.

The American Type Culture Collection includes in its protozoan collection two strains of *Tetrahymena pyriformis.* Because of the difficulty of maintaining and preserving protozoa in culture, a study of freezing and preserving these strains at liquid nitrogen temperatures was undertaken.

*Tetrahymena pyriformis,* R. H. Hall strains H (ATCC 9357) and W (ATCC 10542), deposited in the Collection (*6*) in 1952, were maintained and propagated in tetrahymena broth (5 g of Difco proteose peptone, 5 g of tryptone, and 0.2 g of $KH_2PO_4$ in 1 liter of water). The cultures were grown at 26°C for 50 to 60 hours. Cells for experiments were obtained by pipetting 1 ml from the upper portion of a 7-ml broth culture. Samples to be frozen were usually collected from several cultures. Five-tenths milliliter of dimethylsulfoxide, $(CH_3)_2SO$ (*7*), was added to 4.5 ml of cell suspension, thus producing a 10 percent solution, by volume, of the protective agent.

Flat-bottomed, soft-glass vials (14 by 85 mm) were used to contain the samples. A flat-bottomed vial was selected so that the cell suspension would form a layer of even thickness.

An upright deep freezer (household model) was used for the initial stage of the two-step freezing procedure. This freezer had an air temperature between −17° and −20°C and a shelf temperature of −20° to −23°C. When the specimens were placed in the freezer, the door was opened and closed rapidly so that relatively constant temperatures were maintained.

The temperatures of the air, shelf, and specimen were recorded on a four-point Honeywell Brown Electronik recorder (*8*) throughout the cooling, and the cooling rates were determined from the specimen temperatures recorded by means of a thermocouple inserted into one of the sample vials.

In the second step of the freezing process a liquid nitrogen bath was used to lower the specimen temperature from −20°C to −196°C. For long-term storage of the specimens a liquid nitrogen refrigerator (*9*) was used.

After the protective agent (dimethylsulfoxide) was added to the culture the mixture was incubated at 26°C for 30 minutes. A sample of the suspension (0.2 ml) was then pipetted into each vial. The vials were plugged with cotton but not sealed, and were placed upright on the shelf of the freezer for 20 minutes. At the end of this time the first stage of freezing was complete, and three vials were removed at random from the freezer and the material was thawed immediately. The remaining vials were plunged directly into liquid nitrogen. After 10 minutes in liquid nitrogen several specimens were thawed, and the others were stored in the gas compartment of the liquid nitrogen refrigerator where the temperature was not above −170°C.