Effects of rapid changes in salinity on the renal function of a euryhaline teleost

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FLEMING, WARREN R., AND JON G. STANLEY. Effects of rapid changes in salinity on the renal function of a euryhaline teleost. Am. J. Physiol. 209(5): 1025-1030. 1965.—Techniques developed to study renal function of the euryhaline teleost, Fundulus kansae, are described. Effects of a sudden transfer from fresh water to sea water on urine flow and urine and serum osmotic pressures were studied. Glomerular filtration rates (GFR) of animals adapted to fresh water and to sea water for 7 days were estimated. Urine flow was found to be a function of size and of handling, as well as of salinity. Animals held in fresh water showed an initial diuresis which reached a peak approximately 2 hr after cannulation. Urine flow thereafter was in the range of 200 ml/kg per day for fish weighing 1.58 ± 0.3 g, and the estimated GFR was 600 ml/kg per day. Animals held in sea water had urine flows ranging from 5 to 20 ml/kg per day, and filtration rates ranging from 20 to 45 ml/kg per day were estimated. The urine collected from the 2nd to approximately the 10th day after a sudden transfer into sea water was hypertonic to the serum. It is concluded that both a reduction in GFR and an increase in the tubular reabsorption of water are elements of renal function utilized when this animal moves from a hypotonic to a hypertonic environment.

Little information is available concerning the nature of the changes in renal function which take place when a euryhaline teleost moves from fresh water to a marine environment, or vice versa. It has long been recognized that such fishes must have the ability to alter urine flow, and it has been suggested that such changes could be achieved, at least in part, by changes in the glomerular filtration rate (10, 16). This suggestion has been substantiated only recently for the euryhaline teleosts Salmo gairdneri (8) and Anguilla anguilla L. (15).

The role played by the kidney in achieving and maintaining osmotic homeostasis in the plains killifish, Fundulus kansae, has been a subject of continuing study in this laboratory for a number of years (17, 18). We have been particularly interested in examining the mechanisms which permit this teleost to move rapidly from fresh water to sea water, to survive in extremely hypertonic saline solutions (3), and to return from such environments to fresh water. This paper reports on the techniques developed, on the effect of size and handling on urine flow, on the effect of adaptation to fresh water and sea water on urine flow and glomerular filtration rates, and, finally, on the changes in blood and urine osmotic pressures that are seen when this teleost is suddenly transferred from fresh water to sea water.

METHODS

The fish were collected from a salt spring, "Boonslick," located in Howard County, Missouri. The routine handling of the animals, the methods used to obtain urine and serum samples and to estimate urine excretion, and the analytical methods employed have been described elsewhere (18).

Details of one of the experimental units used are shown in Fig. 1. The design of the glass holding chamber is such that the fish can neither turn around in the tube nor swim through it. The rear end of each tube is partially closed off by a machined Plexiglas block which serves as one point of alignment for the chamber; thus, the fish cannot back out of the chamber and is restricted to limited movement in, essentially, a single plane.

Usually 14 glass chambers are mounted into slots machined in a Plexiglas block, which, in turn, is sealed into a small aquarium made from the same material. The aquarium is divided into two subunits by a center partition. Two large holes through the partition connect the two compartments. By plugging the holes it is a simple matter to change the conditions in one subunit, i.e., by adding salts, drugs, etc., without disturbing the other compartment. The aquarium contains 5 liters of water, and adequate aeration and mixing are provided by eight air stones. The experiments were carried out at 20 ± 1 C.

During our initial experiments, carried out using clear-glass chambers, it became obvious that the fish were disturbed by the ordinary laboratory routines, and many succumbed in getting free of their cannulae. These
difficulties were circumvented by dipping the anterior two-thirds of each chamber into green Tygon paint. This water-insoluble, nontoxic plastic paint was layered on until the dipped portion was opaque to light and provided cover for the animals. Our experience has been that the cannulated animals will usually quiet down within a few minutes after being placed into the dark tubes, and will remain quiet and hidden for the duration of the experiment—120 hr in some cases.

We also used the anesthetic MS222 (Sandoz) in our initial experiments. The supposition was that it would be easier to stitch the cannula into place if the animals were under light anesthesia. It was found, however, that many fish tended to thrash about while recovering from the anesthetic and often tore their cannula loose in the process. Accordingly, it has been our practice to immobilize the animals by wrapping them in damp tissue paper, leaving the urinary papilla and anal fin exposed. Fish treated in this manner can be cannulated quickly and usually remain quiet. After some practice it has become routine to capture, weigh, immobilize, cannulate, and, finally, to place a fish into the glass holding chamber—all in 3 min.

The following method has been used to arrive at an estimate of the glomerular filtration rate. After cannulation, each fish was given a single intraperitoneal injection of C\textsuperscript{14}-labeled inulin solution. Each animal received 0.225 mg of inulin/g body wt carried in a volume of 6.67 ml of deionized water. This volume of solution contained 0.25 \textmu C of isotope. A micrometer-driven syringe was used to deliver the precise volume of inulin solution. Urine was collected at desired intervals (fresh water) or whenever possible (sea water) and 5-ml samples were spotted onto a disk of filter paper for assay. After each sample of urine had been collected, three animals were removed and serum samples prepared for counting in the same way. Thus, urine and serum samples were available from the same animal. The samples were assayed using a thin-window (Micro-mil) gas-flow counter. Thus a urine/serum ratio was measured, and, knowing the rate of urine excretion, a glomerular filtration rate (GFR) could be calculated.

It was noted that urine flow rose sharply after feeding and that the response lasted several hours. Therefore, food was withdrawn 24 hr prior to the start of an experiment.

RESULTS

A typical pattern of urine excretion by cannulated \textit{F. kansas} adapted to fresh water is shown in Fig. 2. The initial rate of urine flow measured was always quite high, and a diuretic peak was always seen, usually within 2 hr after cannulation. Recovery from diuresis was rapid and was usually complete in 7 or 8 hr. The rate of urine flow then oscillated about an average value for days.

As shown in Fig. 3, the GFR's of fresh water adapted fish were quite high. Filtration rates close to 800 ml/kg per day were usually seen shortly after cannulation. The
filtration rate soon fell to about 600 ml/kg per day, and remained fairly constant thereafter.

*F. kansae* could adjust urine production very rapidly when switched from a fresh-water to a sea-water environment. As shown in Fig. 4, a new and steady rate of urine production was reached in a few hours.

A comparison of urine flow and filtration rates of fish held in sea water for 7 days prior to cannulation is shown in Fig. 5. The filtration rate was reduced to 3–8%, and the volume of urine excreted was reduced to 2–5% of the fresh-water values. The initial diuresis seen in fresh water-adapted animals was not evident; in fact, usually no urine could be collected for the first 2 hr after cannulation.

Figure 6 is a composite of a number of experiments which compare the urine and serum osmotic pressures of fresh water-adapted animals that were subjected to a sudden transfer into sea water and then held in that environment for various periods prior to cannulation. Serum-hypertonic urine could be demonstrated after the animals had been in sea water for 2 days. The urine remained hypertonic to the serum for several days. The urine of animals held in sea water for 20 days was isotonic or slightly hypotonic to the serum (17). As shown in Fig. 6, the osmotic pressure of the serum rose sharply after a sudden transfer into sea water and then returned slowly to the levels measured in fresh water. The relationship between the osmotic pressure of the serum and urine of fresh water-adapted animals and of animals undergoing adjustment to sea water is shown in Fig. 7. It appears that a serum-hypertonic urine was not excreted once the osmotic pressure of the urine fell to about 400 milliosmols/liter.

The diuretic peak seen in fresh water-adapted animals suggested that handling affects urine flow in *F. kansae*. Another factor that was important was size, and the relationship between body weight and urine flow is shown in Fig. 8. The slope of the least-squares regression line was different from zero ($P = 0.001$). This relationship largely disappeared during the diuretic period, and the slope of the regression line obtained from data taken during this period showed only borderline significance.

**DISCUSSION**

Although the absolute values differ considerably, the data presented here agree, in a general sense, with that reported from other laboratories. Thus Sharratt et al. (15) report that the GFR of eels held in sea water is about 20% of the fresh-water values. Holmes and McBean (8) report a 93% drop for the rainbow trout held in 80% sea water. Our data suggest a 92–97% decrease for *F. kansae* held in sea water for 7 days. Thus, it seems certain that the low rate of urine production in sea water is due in part to a marked reduction in GFR.

Tubular reabsorption of water is well known in marine and fresh-water stenohaline teleosts (2, 5, 11–14) and the available data show the same capability in euryhaline teleosts. Sharratt et al. (15) report that the eel reabsorbs approximately 24 and 40% of the volume filtered in fresh water and sea water, respectively. Our data show that from essentially zero (peak diuresis) to about 60% of the filtered load is reabsorbed in fresh-water animals, and that about 70–80% is reabsorbed in sea-water animals. Holmes and McBean (8) combine their data with those of Holmes (7) and conclude that the rainbow trout reabsorbs 42–53% of the filtered load in fresh water, and from 90 to 95% in sea water. Hunn and Fromm (9) also combine data (with Holmes and McBean, 8) and conclude that the rainbow trout reabsorbs from 40 to 70% of the filtered urine in fresh water.

The data presented in Fig. 4 suggest that *F. kansae* can activate both mechanisms quite rapidly.

We wish to emphasize that the techniques used here
to estimate the GFR require that urine be collected at relatively short intervals, and that fish be removed from the aquarium every few hours in order to obtain blood samples—at least in the experiments carried out in fresh water. Such procedures are necessary because *F. kansae* clears inulin very rapidly. Thus the fish are necessarily disturbed quite often and undoubtedly remain somewhat diuretic throughout the entire experimental period. The data in Fig. 3, however, suggest that the filtration rate reaches a fairly steady value within 2 hr after cannulation, and that much of the diuresis is due to a low rate of tubular reabsorption of water.

The glomerular filtration rates reported here are quite high when compared with the available data from other euryhaline teleosts held in fresh water. Sharratt et al. (15) report a mean GFR of 110 ml/kg per day for the eel, and Holmes and McBean (8) report a mean figure of 157 ml/kg per day for the rainbow trout. Our data show a GFR of 800 ml/kg per day during the diuretic peak, and a relatively steady value of 600 ml/kg per day thereafter. This could mean that our animals remained extremely diuretic throughout the experimental period—and we have not completely discounted this possibility. We feel, however, that much of the difference can be explained on the basis of size. The fish used by Holmes and McBean (8) weighed 186 ± 5.9 g, the eels used by Sharratt et al. (15) weighed 178 ± 58 g. The animals used here weighed 1.58 ± 0.3 g. There would be, then, a considerable difference in the body weight/surface area ratios of our animals and the heavier fish, and probably of even more importance, a considerably larger proportion of the body surface would be made up from water-permeable surfaces, i.e., gills and oral membranes. Figure 8 shows that urine flow and presumably, therefore, water permeability is definitely a function of body weight.

Figures 6 and 7, which compare the serum and urine osmotic pressures of *F. kansae* at various intervals after transfer into sea water, extend and confirm data published earlier (18), wherein it was reported that this killifish could under certain conditions excrete a blood-hypertonic urine. Such results were not expected, and are contrary to those reported for other teleosts. It seems pertinent, then, to consider the possibility that our data may be due to undetected errors.

An obvious source of error could be the undetected leakage of a small amount of sea water into a cannula, thereby increasing the osmotic pressure of urine that was actually isotonic or hypotonic at the time it was excreted. Our experience has been that if a cannula is not properly fixed, a leak will develop usually within the first few hours of an experiment. Such leaks are easy to detect in sea water, for one can observe a steady increase in urine volume over a relatively short period of time. If the cannula is properly fixed, urine is excreted erratically, i.e., the volume of urine present in the tubing will often remain constant for 1 or even several hours. A leak is even easier to detect in fresh water, for the volume of fluid in the cannula will rise only to the level of the water surface, and remain at that point.

Another possible source of error could be founded in our assumption that the comparison of the osmotic pressure of urine—which had been collected over a period of hours—with that of the serum which is prepared after
the urine sample is taken is a valid one, i.e., the possibility remained that the osmotic pressure of the urine really reflects the osmotic pressure of the serum some hours or even days previously. To check on this possibility, we made use of the indwelling catheter technique introduced by Maetz (11). Five microliters of phenol red solution were introduced into the peritoneal cavity of fish that had been cannulated and placed into sea water 2 days previously. In every case, the dye appeared in the collection tubing within 12 hr. Thus, we assume that we are dealing with a lag period of approximately 12 hr. Further evidence against a long lag period is given in Fig. 6, where it may be seen that the peak values measured for the urine are well above those measured for serum and remain so for days.

We conclude, then, that our claim that _F. kansae_ can excrete a blood-hypertonic urine is a valid one.

The nephron unit of this killifish consists of a glomerulus, neck segment, proximal convoluted segment which may be divided into two histologically distinct portions, initial collecting segment, and collecting tubule. Thus it is typical of marine teleosts and similar to that of _F. heteroclitus_ (3, 5). Certainly nothing obviously comparable to the loop of Henle can be demonstrated. Since we do not at this time have any information about the mechanism or mechanisms that _F. kansae_ uses in order to excrete a hypertonic urine, and cannot say with any certainty why the animal soon ceases to do so, we are left at this point with a number of speculations which need not be pursued here. It should be pointed out that although our evidence does show that under certain conditions it is possible to collect a blood-hypertonic urine, it does not force us to the conclusion that the kidney is the site of formation of such urine. Such a conclusion would require that urine be collected from the ureters rather than from the bladder—a requirement that is, at least at this time, beyond our capabilities.

Finally, it should be pointed out that in a teleological sense at least, the ability to excrete a hypertonic urine would seem to serve no useful purpose, i.e., the urine excreted is still hypotonic to the environment. Thus, as Forster (5) has already emphasized, the kidney of marine teleosts can be regarded as a liability since it adds to the osmotic difficulties of the animal.
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REFERENCES