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# GLOMERULAR FILTRATION RATE AND RENAL TUBULAR SECRETION OF FLUID IN THE EEL *(Anguilla rostrata)*

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The American eel, *Anguilla rostrata*, is a euryhaline catadromous species. The adult silver form spawns in the sea, and the immature yellow form inhabits fresh water. The present study is concerned with the normal function of the kidney of fresh water-and sea water-acclimated yellow eels. The investigation was undertaken as a part of a long-term program to study the effects of heavy metals on osmoregulatory functions of marine and fresh water fishes.

Commerically obtained eels weighing 58 to 225 gm were maintained at least three weeks in either flowing sea water or flowing fresh water prior to experimentation. The temperatures of the acclimation media were moderately variable (sea water 8°-15° C; fresh water 11°-14°). While anesthetized (MS-222, ca. 1:2000 w/v) a retention catheter of PE 60 tubing (Intramedic) was placed in the uropore and fastened with a purse string suture. In addition, PE 10 tubing was inserted into the hemal vein near the caudal end of the fish and sutured in place. The fish were then placed inside plexiglass chambers to restrict excessive movement. The chambers were equipped with flowing sea water or fresh water. The fish were allowed to recover from the catheterization procedure at least 24 hours before experimentation.

Glomerular filtration rate (GFR) was determined with both polyethylene-1,2-$^{14}$C glycol (PEG-$^{14}$C) and inulin methoxy-$^{3}$H (New England Nuclear Corporation). Each animal was given 25 $\mu$l of a solution containing 2.5 $\mu$Ci of PEG-$^{14}$C per ml of 0.7% NaCl and 25 $\mu$l of a solution containing 100 $\mu$Ci of inulin-$^{3}$H per ml of 0.7% NaCl via the venous catheter. This catheter was subsequently rinsed with the animal's own blood at least five times and filled with heparinized saline. After a six hour distribution time for the injected material, urine and blood sampling were begun. In most eels 5 to 7 urine samples were collected and 6 to 8 blood samples. Each urine collection period usually lasted 24 hours.

PEG clearance was consistently higher than the inulin clearance by approximately 25% (Table 1). This finding is in agreement with data in rats by Burglund et al (Acta Physiol. Scand. 76:458-462. 1969) who found the clearance ratio between PEG and inulin-$^{3}$H to be 1.44. They concluded that the lower inulin clearance was due to incomplete filtration of the inulin molecules.

The rate constants for the excretion of the two substances (Table 2) were calculated from the exponential decrease in the plasma concentration in each eel. Two examples are shown in Figure 1. Calculations of the body clearance can be made from these rate constants when accurate determination of the distribution volumes for each of the two substances have been made. So far it appears that the body clearance exceeds the renal clearance.

Table 1 shows that GFR of sea water-acclimated eels was not statistically significantly different from that of fresh water acclimated eels. The urine flow was significantly higher in fresh water than in sea water-acclimated eels.

In the fresh water-acclimated eels the average PEG U/P ratio was 1.19 (Table 1). In 19 out of 43 urine collection periods the PEG U/P ratios (and inulin U/P ratios) were lower than unity; i.e.,

TABLE I

| | Seawater Eels | Freshwater Eels |
|---|---|---|
| GFR(PEG) ml/kg.hr | 3.05 ± 0.64 S.D. 2.40 (14) | 2.27 ± 0.30 S.D. 1.96 (42) |
| U/P (PEG) | 4.87 ± 0.75 * S.D. 2.94 (15) | 1.19 ± 0.13 S.D. 0.88 (43) |
| Urine flow ml/kg.hr | 0.83 ± 0.13 * S.D. 0.50 (20) | 1.87 ± 0.21 S.D. 1.41 (45) |
| $\frac{\text{PEG clearance}}{\text{Inulin clearance}}$ | 1.14 ± 0.08 S.D. 0.22 (15) | 1.29 ± 0.05 S.D. 0.39 (42) |

* $P < 0.001$

TABLE 2

RATE CONSTANT (k) FOR EXCRETION OF PEG AND INULIN IN % $HR.^{-1}$

| | Seawater Eels | Freshwater Eels |
|---|---|---|
| $k_{PEG}$ | 3.50 ± 0.41 S.D. 0.82 (4) | 2.29 ± 0.42 S.D. 1.32 (10) |
| $k_{Inulin}$ | 2.80 (2.07 - 3.55) (2) | 1.46 ± 0.24 S.D. 0.76 (10) |
| $\frac{k_{PEG}}{k_{Inulin}}$ | 1.21 (1.12 - 1.31) (2) | 1.57 ± 0.35 S.D. 1.1 (10) |

TABLE 3

URINE OVER PLASMA RATIOS FOR PEG AND INULIN IN CONSECUTIVE SAMPLES FROM EIGHT FRESHWATER EELS

| | PEG U/P | Inulin U/P | | PEG U/P | Inulin U/P |
|---|---|---|---|---|---|
| Eel 25 | .77 | .60 | Eel 33 | 1.25 | .99 |
| | .55 | .34 | | 1.15 | .78 |
| | .30 | .26 | | .70 | .62 |
| | .40 | .33 | | .54 | .49 |
| | .46 | .38 | | .19 | .21 |
| | | | | .03 | .08 |
| Eel 27 | 1.28 | .95 | | | |
| | 1.67 | 1.37 | Eel 34 | .41 | .28 |
| | 1.96 | 1.48 | | .35 | .31 |
| | 1.83 | 1.42 | | .17 | .16 |
| | .62 | .58 | | .09 | .12 |
| | | | | .30 | .28 |
| Eel 31 | 1.86 | 1.28 | | .39 | .35 |
| | 1.70 | 1.19 | | | |
| | 1.53 | 1.06 | Eel 35 | 1.76 | 1.46 |
| | 1.09 | .81 | | 1.43 | 1.11 |
| | 1.14 | .65 | | .91 | .70 |
| | | | | 1.26 | .99 |
| Eel 32 | 1.72 | 1.17 | | .73 | .57 |
| | 1.72 | 1.23 | | .42 | .33 |
| | 2.01 | 1.46 | | | |
| | 1.97 | 1.45 | Eel 36 | 1.58 | 1.35 |
| | | | | 1.60 | 1.30 |
| | | | | 1.66 | 1.25 |
| | | | | 2.24 | 1.51 |
| | | | | 3.25 | 1.63 |
| | | | | 4.33 | 1.75 |

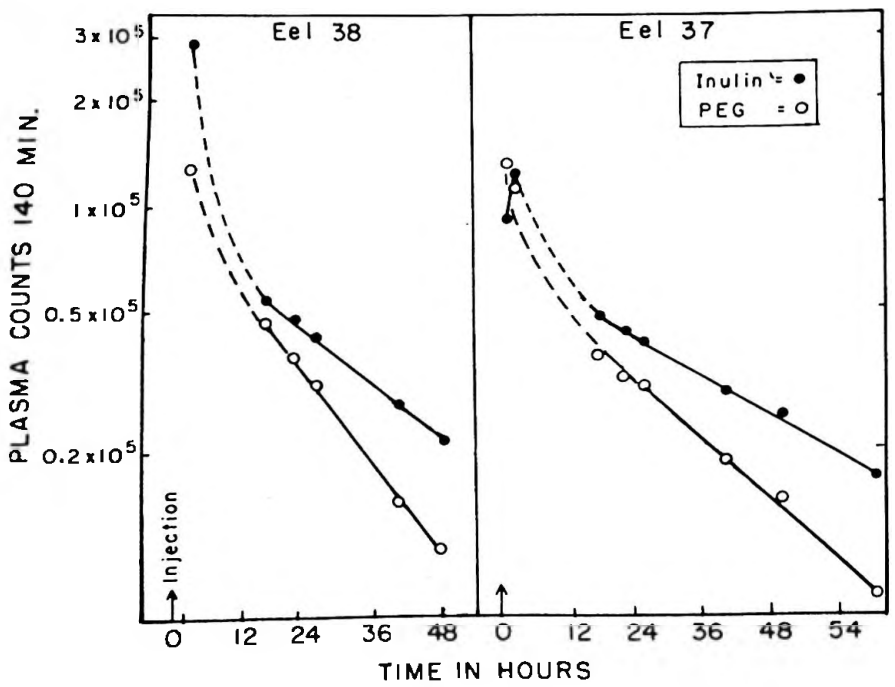


Figure 1

either urine flow was greater than the GFR, indicating renal tubular fluid secretion, or inulin and PEG were reabsorbed. In none of the sea water-acclimated eels did the urine flow exceed the GFR. In both groups of eels the urine was osmotically dilute. The average free water clearance ($C_{H_2O}$) was 1.57 ml/kg hr. in fresh water and 0.41 ml/kg hr. in sea water-acclimated eels. The difference in $C_{H_2O}$, (1.15 ml/kg hr), corresponds closely to the difference in urine flow between the two groups, 1.04 ml/kg hr.

Aglomerular urine secretion is typical of the aglomerular marine teleosts. It has, however, been observed sporadically in glomerular teleosts, e.g., Grafflin (J. Morphol. 61:165-173, 1937) and Hickman (Canadian J. Zool. 46:427-437, 1968). It is unusual that it should be found in the fresh water- rather than in the sea water-acclimated eels.

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## RENAL COLLECTING DUCT FUNCTION IN THE LITTLE SKATE, *Raja erinacea*.

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The renal collecting ducts of the skate originate segmentally on the dorso-lateral surface of the kidney and receive the terminal portions of nephrons from each segment. The ducts turn, coursing medially between lobules, and enter the kidney substance at its dorso-medial aspect. Emerging at the ventral surface the ducts enter the urinary sinus, either directly or after joining the other tributary ducts. The collecting ducts are visible and accessible to micropuncture throughout their dorsal surface portions.

We have examined fluid taken from early and successively more distal portions of the collecting ducts for osmolality and electrolyte composition ($Na^+ + K^+$) and compared these to more proximal tubular fluid and to final urine. In some experiments collecting ducts were filled retrograde with colored microfil and the more distal surface tubules visualized. The collected fluid was analyzed for osmolality by the freezing point method of Ramsay-Brown and some samples for sodium and potassium concentration by the microflame photometer of Hampel. (We are indebted to Dr. Bodil Schmidt-Nielsen for the use of this equipment and to Mr. Yogendra who did the flame photometry).

Studies were completed on 20 male and female skates weighing from 0.88 to 1.59 kg and anesthetized by injection of 0.45 mgs/kg sodium pentobarbital and 0.45 kgs/kg curare into a lateral tail vein. Fish were placed on a board dorsal or ventral side up, the spiracles perfused with aerated sea water (100 or 75% at 1 - 1.5 L/min. thermostated at 10°C.) A paramedian incision exposed the dorsal or ventral surface of the kidney for direct visualization. To minimize interference with the renal blood supply, the tail was not fixed. The exposed surface of the kidney and surrounding tissue were covered with a layer of 2% liquid agar which hardened in a few minutes. The site of micropuncture was then exposed by a small window cut in the agar. Samples were aspirated through sharpened micro glass capillaries ground to a tip diameter of 8-15 $\mu$. The appearance of the area of the puncture site is shown in Figure 1, after retrograde injection of microfil. The collecting ducts,