

of Love (Gut 10:105-107, 1967) but that disagrees with the work by Lifson et al (Am. J. Physiol. 233:1479-1487, 1972) and Rohde et al. (Gut 13:191-196, 1972). From the limited amount of data presented here one may speculate that the CT has a similar action on flounder intestine to that observed with mammalian intestine and that SEB acts in a similar fashion.

This work was supported by grants from NSF GB-27495 and NIH AM-2217-14. Cholera toxin prepared under contract for the NIAID by R.A. Finklestein, PhD, The University of Texas Southwestern Medical School, Dallas, Texas.

1972 #45

RENAL UREA AND WATER REABSORPTION IN THE LITTLE SKATE *Raja erinacea*

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In our studies of renal excretion of urea in the dogfish *Squalus acanthias* (Bodil Schmidt-Nielsen, et al. Comp. Biochem. Physiol. 42A:13-25, 1972), a constant relationship between urea and sodium reabsorption was found. Thus $1.6\mu\text{M}$ urea is reabsorbed per μM of Na reabsorbed. The present studies with the little skate *Raja erinacea* were undertaken to determine if this same relationship exists in another species of elasmobranch.

We performed preliminary dissections to determine the position and anatomical arrangement of ureters and urinary vesicle. The anatomy in *R. erinacea* was found to be quite similar to that described by I. Borcea in *R. clavata* (Arch. Zool. Exp. et Générale IV Série, 4:199-484, 1906). In the adult female skate the bilobed urinary vesicle is a diverticulum to the urogenital sinus. It opens through a single canal with a sphincter into the urogenital sinus between the openings of the two oviducts. Four to five ureters from each kidney open into the dorsal side of each of the two lobes of the urinary vesicle. The anatomical arrangement in the male is different but also in the male there are distinct urinary vesicles. We chose to work with the female for the present study.

Twelve adult female skates, *R. erinacea*, were used-body weight 800 - 1000 g. Some of the fish were acclimated to 75 percent seawater for two days prior to the experiment. The skate was anesthetized by superfusion of the gills with a one percent MS 222 solution. A 2 cm incision was made in the skin of the abdomen and a 1 cm incision in the wall of the urogenital sinus. A polyethylene catheter was inserted through the opening into the urinary vesicle and tied in place with a pursestring ligature. The incisions were sutured tightly. Apparently this procedure is not very traumatic to the skate since they could live for several weeks with the catheter in place. The catheter could be irrigated with air through a side tube so that the bladder and catheter could be effectively emptied at the end of each urine collection. ^{14}C labelled polyethylene glycol was used for determination of glomerular filtration rate. The isotope was injected into the caudal vein from eight to 24 hours prior to the start of the experiment. Urine collection periods varied between two and 17 hours. In some experiments trimethylamine oxide (20 ml of 5M TMAO per kg fish) was injected i.v. and in other experiments a similar dose of urea was given. The purpose of these injections was to see if a relative change in the plasma concentration of TMAO or urea would change the ratio of

TABLE 1
COMPARISON OF RENAL FUNCTION IN
R. erinacea AND *S. acanthias*

	<i>R. erinacea</i>	<i>S. acanthias</i>
In Seawater		
GFR ml/Kg.hr.	0.43 ± 0.09 (9)	1.72 ± 0.16 (21)
Osmolar U/P	0.98 ± 0.03 (9)	0.778 ± 0.02 (35)
$\dot{Q}_{\text{urea reab.}}/\dot{Q}_{\text{Na reab.}}$	1.58 ± 0.16 (9)	1.60 ± 0.03 (35)
$C_{\text{H}_2\text{O}}/\text{GFR}$	0.025 ± 0.007 (9)	0.075 ± 0.008 (34)
In 75% Seawater		
GFR ml/Kg. hr.	0.45 ± 0.05 (5)	5.86 ± 0.54 (21)
Osmolar U/P	0.78 ± 0.05 (5)	0.506 ± 0.018 (21)
$\dot{Q}_{\text{urea reab.}}/\dot{Q}_{\text{Na reab.}}$	1.20 ± 0.25 (5)	1.60 ± 0.03 (19)
$C_{\text{H}_2\text{O}}/\text{GFR}$	0.20 ± 0.07 (5)	0.201 ± 0.010 (19)

Values given as mean ± S.E.
Figures in parentheses indicate numbers of samples

tubular urea to Na reabsorption.

In Table 1 the average results of renal function in *R. erinacea* are compared with our previous results in *S. acanthias*. The GFR was the same in seawater and 75 percent S.W. skates in these experiments. This finding is different from that of Goldstein and Forster (Amer. J. Physiol. 220:742-746, 1971) who found an increase in GFR in 50 percent S.W. acclimated fish. The free water clearance was increased in the 75 percent S.W. acclimated skates and the osmolar U/P was reduced.

The average of the ratio $\mu\text{M urea reab.}/\mu\text{M Na reab.}$ was the same in S.W. skates as in *S. acanthias* but the average ratio was lower: 1.2 in 75 percent S.W. acclimated skates. When the data are plotted against fractional Na reabsorption it is seen that the ratio in S.W. acclimated skates decreases with increasing fractional Na reabsorption (Figure 1). In 75 percent S.W. acclimated skates the ratio is lower but it also here decreases with increasing fractional Na reabsorption (Figure 2). In *S. acanthias* on the other hand no trend of this kind is seen in the range of Na reabsorption between 55-95 percent (Figure 3).

The skate data indicated that urea reabsorption might be dependent on water reabsorption and consequently the data were plotted as fractional urea reabsorption versus fractional water reabsorption (Figure 4). The data gave a straight line $y = 0.491 + 0.725X$. The intersect with the y axis indicates that at no water reabsorption, 49 percent of filtered urea is reabsorbed. The points for negative water reabsorption were obtained primarily after urea administration. They indicate that in the skate tubular fluid can be secreted in addition to being filtered. The line intersects the x axis at -0.69. The correlation coefficient for the fit is 0.88.

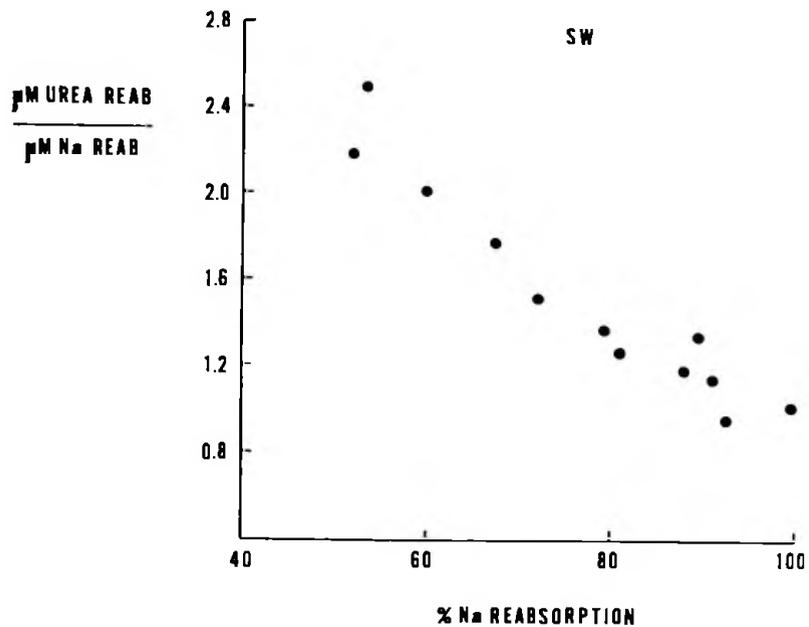


Figure 1. *Raja erinacea* S.W. acclimated. Ratio of urea reabsorbed over Na reabsorbed plotted against fraction of filtered Na reabsorbed on the abscissa.

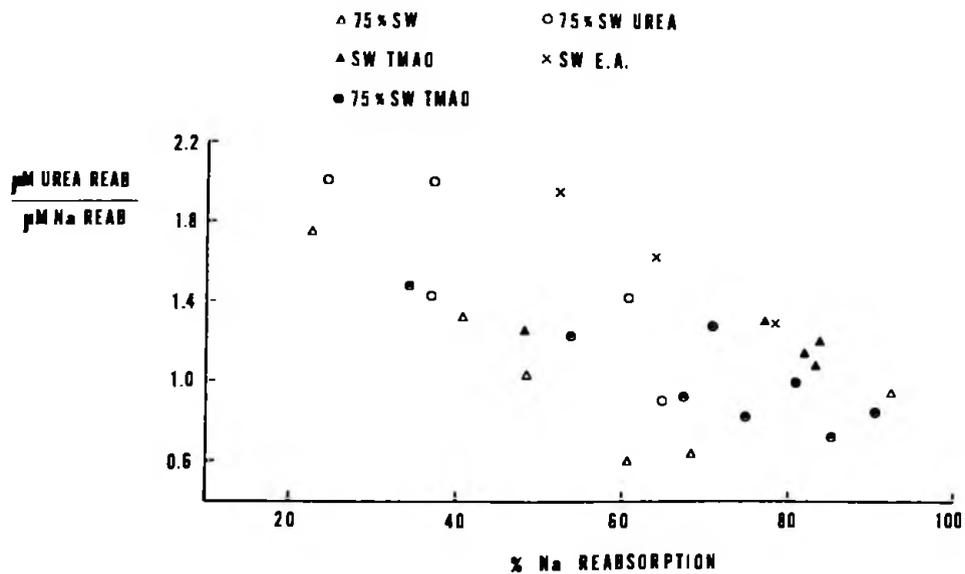


Figure 2. *Raja erinacea*. S.W. and 75 percent S.W. acclimated fish. Some have been injected with TMAO, Urea or Ethacrynic acid (E.A.).

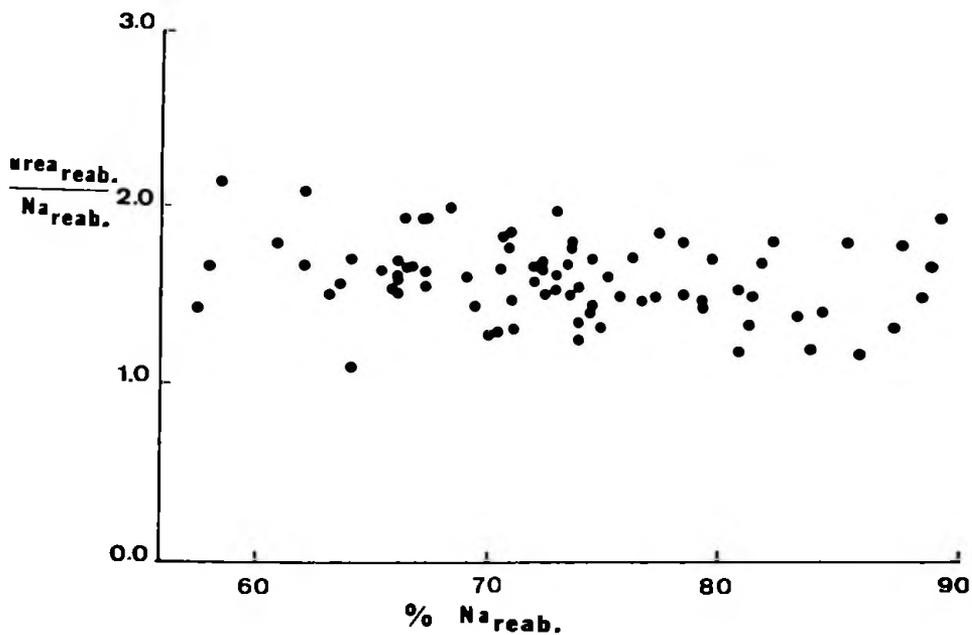


Figure 3. *Squalus acanthias*. Data replotted from Schmidt-Nielsen et al. Bull. MDIBL 6:35-36, 1966).

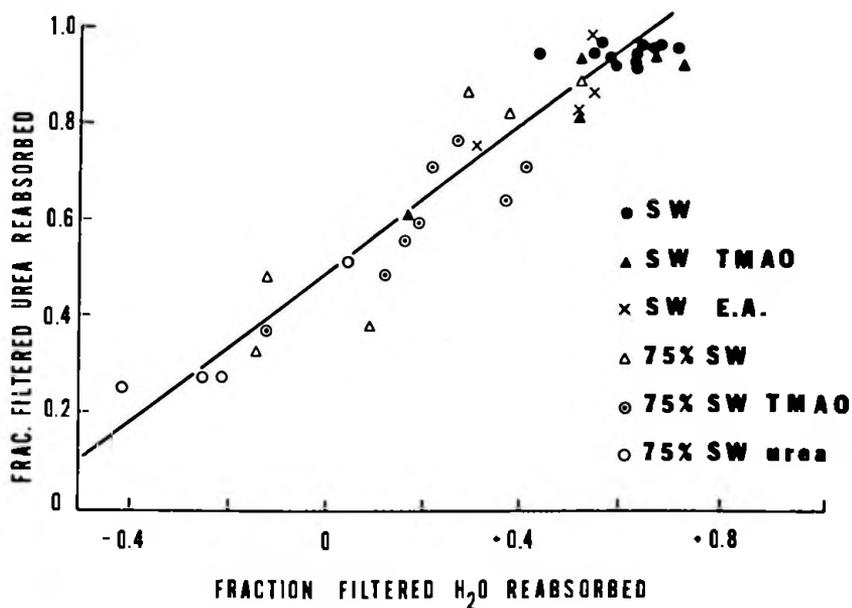


Figure 4. *Raja erinacea*. Fraction of filtered urea reabsorbed (ordinate) plotted against fraction of filtered water reabsorbed (abscissa).

If the findings are correct (we need further verification concerning fluid secretion) the interpretation of the findings could be that fluid is added to the tubule at all times in an amount corresponding to about 70 percent of the amount filtered. That the little skate secretes fluid into the tubules is consistent with the finding that other glomerular fishes secrete fluid (Marshall & Grafflin, *J. Cell and Comp Physiol.* 1-2:161-176, 1932-33; Hickman, *Can. J. Zool.* 46:427-437, 1968; and Renfro and Schmidt-Nielsen, *Fed. Proc.* 31:344, 1972).

We may reasonably assume that fluid added to the tubule has a very low urea concentration since the rectal gland excretes a fluid with a urea concentration of only 40 mM (Burger & Hess, *Science* 131:670-671, 1960). Electrolytes, fluid, and urea could be reabsorbed together in the long proximal tubule in a solution almost identical to the tubular fluid. This makes sense (since micropuncture studies have shown that the osmolality and urea concentration are almost the same as in the plasma along the entire proximal tubule) (Schmidt-Nielsen et al., *Bull. MDIBL* 6:35-36, 1966). The micropuncture studies in *S. acanthias* have shown that between the proximal tubule and the collecting duct the tubular fluid becomes dilute and the urea concentration decreases. This may be accomplished either (1) by addition of an isosmotic solution to the tubule with subsequent reabsorption of electrolytes or (2) by addition of a dilute solution. It is possible that the anatomical arrangement of the tubule described by Deetjen, et al., (*Bull. MDIBL* 10:5-7, 1970) serves to create a dilute environment around the tubule promoting diffusion of water into the tubule. In *R. erinacea*, it has been found by Stolte, et al. (*Bull. MDIBL* 11:91-93, 1971), that at least part of the dilution takes place in the collecting duct. Thus the data in the little skate could be consistent with the first hypothesis.

In annelids the anatomical arrangement of the nephridium resembles that of the elasmobranch (Boroffka, *Pflügers Archiv.* 281: 25-48, 1964; and Schmidt-Nielsen & Pagel, *Bull. MDIBL* 9: 58-59, 1969). In the leech micropuncture studies of the nephridium of the bladder have shown that all concentrations inside the tubule, osmolality, inulin, sodium, and potassium decreased by about the same factor (1/3) in the last part of the tubule before the bladder (Schmidt-Nielsen and Patel in preparation). This finding is also consistent with the interpretation that fluid is added in the last segment of nephridium before the bladder.

From the data presented above it is conceivable that the elasmobranch renal tubule does not reabsorb urea actively but reabsorbs urea together with electrolytes and water in the proximal tubule. Dilution of the tubular urea concentration is then brought about through tubular secretion of a fluid with a low urea concentration. Further experimentation is necessary to prove this hypothesis.

This work was supported by NIH Grant #AM 15972.