In the segment at the rectal end of the spiral valve, mean was 338 μmoles/ml, S.E. = 7, n = 3.

Bacterial breakdown of urea was studied in the fluid expressed from the intestines of the skate, Raja erinacea and dogfish. In some preliminary studies in the skate, intestines were tied off, removed, and rinsed with an "elasmobranch Ringer's" solution. The fluid expressed was then incubated at 15-18° C for one hour. There was a definite increase in the concentration of ammonia during the hour, and this increase was less marked when antibiotics were added to the expressed fluid. Although these studies were non-quantitative, we may conclude that there is at least some bacterial breakdown of urea in the skate intestine. In preliminary studies in dogfish, fluid was expressed from the intestine and incubated at 13° C for one hour. Samples were taken at 20-minute intervals, precipited in cadmium sulfate, and analyzed for ammonia content. The increase in ammonia during the one-hour period averages 2.17 μmoles/ml, S.E. = 1.96, n = 3. From this figure it can be calculated that approximately 4 μmoles urea/kg fish per hour are degraded in the dogfish intestine. Although small compared to the total amount of urea lost from the fish, these preliminary studies suggest that bacterial degradation of urea must be considered in urea balance studies.

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1969 #17

SALT AND WATER TRANSPORT IN ISOLATED EEL INTESTINE

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Marine teleosts maintain water balance by drinking sea water and absorbing sodium chloride and water across the intestine. One of the physiological changes which euryhaline fish undergo on adaptation to sea water is an increase in the rate of intestinal absorption of salt and water (Skadhauge and Maetz, C. R. Acad. Sc. Paris 265:347-50, 1967) which is mediated by the pituitary-adrenal axis (Hirano and Utida, Gen. and Comp. Endocrin. 11:373-80, 1968). However, the mechanism by which the increase in salt transport is accomplished in the intestine is not clearly understood.

When freshwater eels, Anguilla rostrata, were transferred directly to full strength sea water they died on the third day after transfer, apparently due to their inability to adjust their osmotic and ionic regulatory mechanisms fast enough. This is illustrated in data for blood chloride levels obtained by serial sampling 3 fish (Figure 1). Blood chloride levels rose rapidly for the first 24 hours, then started to level off but all of the fish died on the third day after transfer. However, eels could be preadapted to survive direct transfer to sea water by injection of hydrocortisone (400 μg/100 gm body weight/day) for 2 weeks before transfer and by continuing injections after transfer. When these fish were transferred to full sea water, plasma chloride levels rose rapidly during the first day and then leveled off in the second day and remained constant in 2 of the 3 fish for the remaining 4 days of the experiment (Figure 1). The plasma chloride of the third fish rose slowly over the 6 days of the experiment reaching 185 mM/1 on the sixth day.

All eels used to study sea water adaptation of gut transport were placed in 50% sea water for 2 days before being transferred to 100% sea water. Transfer to sea water was considered to

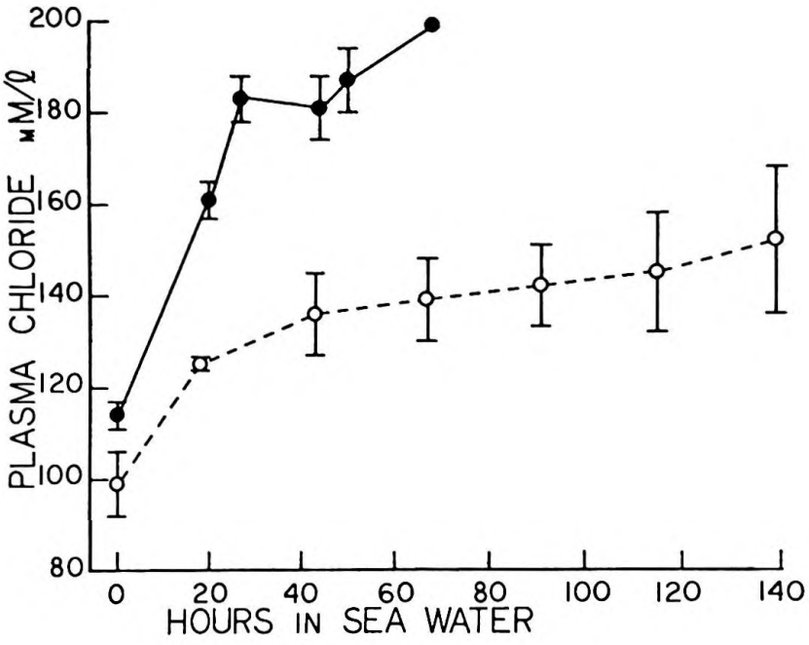

Figure 1. Changes in plasma chloride level on transfer of freshwater eels directly to sea water. Vertical bars represent one standard error of the mean; •, untreated freshwater eels; o, hydrocortisone injected freshwater eels.

have taken place at the time the fish were placed in 50% sea water. In this study water transport was studied in vitro using intestinal sacs bathed on both mucosal and serosal sides with isotonic saline. The sacs were filled with saline, blotted, weighed and placed in saline saturated with oxygen for 1 hour with occasional stirring. After 1 hour the gut was blotted and weighed again. The difference between the initial and final weights was taken to represent the net water transport. The gut was cut open, rinsed, blotted and weighed. Water transport was calculated on the basis of this wet gut weight. Terminal samples of mucosal and serosal fluid were taken for ion analysis. The data for ion transport will be reported elsewhere.

Following transfer to sea water gut water transport increased reaching a maximum on about the third day after transfer (Figure 2), then decreased more slowly reaching a stable level after about 2 weeks in sea water. This phenomena has also been observed in the Japanese eel (Oide and Utida, Marine Biol. 1:102-5, 1968). Water transport was partly inhibited by 10^{-4} M ouabain both in fresh water and in salt water ($\geq$ 14 days) adapted fish (Figure 3). Since the rate of transport in ouabain poisoned guts from fresh and salt water animals was the same, the total increase in transport during sea water adaptation is ouabain sensitive and hence probably represents an increase in sodium transport. A large component of water transport in both fresh water and salt water adapted eels was not inhibited by ouabain. To determine whether any of the observed in vitro transport was an artifact of the preparation, guts from eels which had been in sea water for 3 days were poisoned with 10^{-3} M KCN plus 10^{-3} M Iodoacetic acid (IAA). These two compounds completely inhibited all transport (Figure 3). Injection of freshwater eels with hydrocortisone (400 μg/100 gm/day) for two weeks stimulated gut transport almost to the level observed in eels adapted to sea water for 3 days (Figure 2). This effect of hydrocortisone agrees with the finding of Hirano and Utida in the Japanese eel. Guts from freshwater eels which had received two injections of Depo Medrol (methyl prednisolone) (0.3 mg/100 gm body weight) the first two weeks

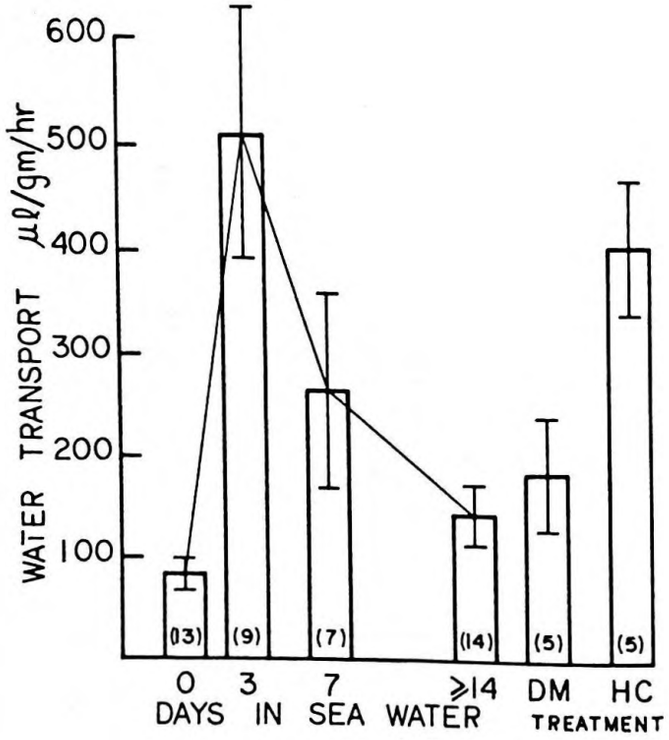

Figure 2. Changes in water transport by isolated eel intestine during sea water adaptation. The two bars on the right show the effect of injection of Depo Medrol (DM) and hydrocortisone (HC) on water transport in intestines from freshwater eels. Vertical bars represent one standard error of the mean. The number of intestines used in each sample is in parenthesis.

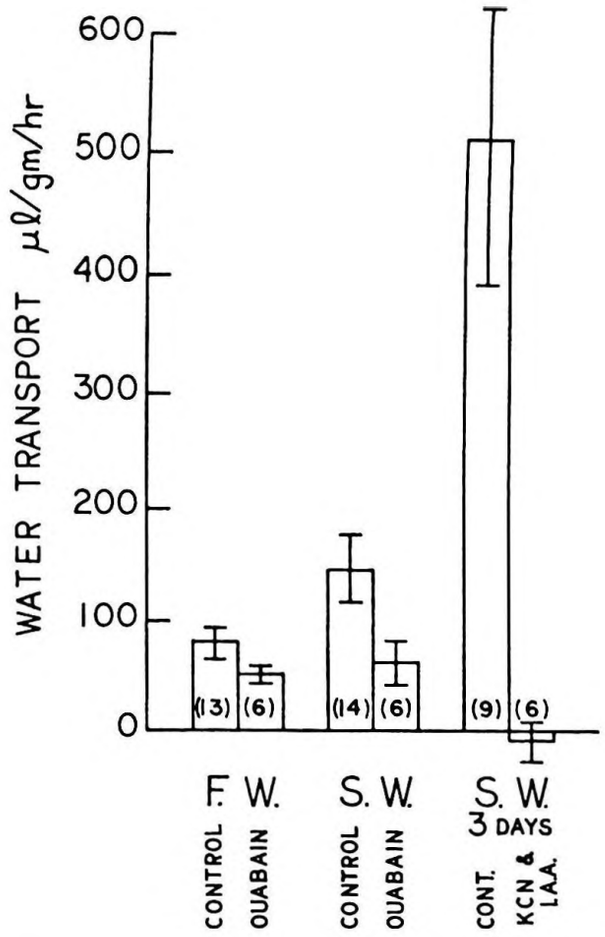

Figure 3. Effect of ouabain on water transport in the isolated eel intestine from freshwater (FW) and sea water (SW) adapted fish, and the effect of potassium cyanide plus iodoacetate on water transport in intestine from eels in sea water for 3 days. The bar on the left of each pair represents transport in untreated control intestines. The bar on the right represents transport in poisoned intestines. The number of intestines used in each sample is in parentheses.

and the second one week before sampling, showed transport rates comparable to sea water adapted fish (Figure 2).

The present studies demonstrate that all water transport observed in vitro in the eel intestine was due to active metabolic processes and that the increase in water transport during sea water adaptation was due to an increase in sodium transport across the gut. However, a large component of water transport is not ouabain sensitive and may be due to the transport of other solutes. Gut water transport in the eel increases sharply during the first few days of sea water adaptation and then decreases to a level approximately twice as high as in freshwater eels. Similar increases in water transport can be obtained in guts from freshwater eels which have received injections of hydrocortisone or Depo Medrol.

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1969 #18

OSMOTIC AND DIFFUSIONAL WATER PERMEABILITY IN TADPOLES AND FROGS, Rana clamitans

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In one of the earliest biological experiments using isotopic tracers (Hevesy, Hofer and Krogh, Skan. Arch. Physiol. 72:199-214, 1935) it was found that the permeability of frog skin to water as measured by the unidirectional diffusion of heavy water, differed from the permeability as measured by net flux of water under an osmotic gradient (osmotic flux). The osmotic permeability of frog skin or (P_f) was found to be greater than the diffusional water permeability (P_d) by a factor of about 5. Later (Koefoed-Johnson and Ussing, Acta Physiol. Scand. 28:60-76, 1953), the conclusion was reached that water flux due to an osmotic gradient takes place as bulk flow through pores.

In the adult frog antidiuretic hormone (arginine vasotocin) greatly enhances the osmotic permeability with little effect on the diffusional permeability (Maetz, in Perspectives in Endocrinology. Barrington and Jorgensen, eds. New York: Acad. Press, 1969). Such a feature is advantageous for the amphibians as it allows them to rapidly take up water through the skin when they return to the pond. In the tadpole arginine vasotocin has only a very small effect on water balance (Alvarado and Johnson, Comp. Biochem. Physiol. 18:549-61, 1966). Furthermore, it has been found that the morphology of the tadpole skin differs from that of the frog and that the structure of the skin changes during metamorphosis (R. E. Taylor, personal communication). For these reasons we expected that the relationship between P_d and P_f in tadpoles might differ from that of the frog. We therefore set out to measure P_d and P_f in tadpoles and recently metamorphosed frogs of comparable size. Diffusional water flux was measured by using tritiated water. The method used was described for the leech (Schmidt-Nielsen and Pagel, Bull. MDIBL 8:61-62, 1968). The urine flow from animals placed in tap water was measured. Since the animals maintain a constant body weight and do not feed or drink during the experiments, the urine flow must equal the osmotic net water influx across the body surface (gills and skin). Diffusional water flux expressed as milliliters of net influx per kg body weight per hour (under the osmotic gradient existing between tap water and body fluid osmolalities) was significantly greater in 7 tadpoles