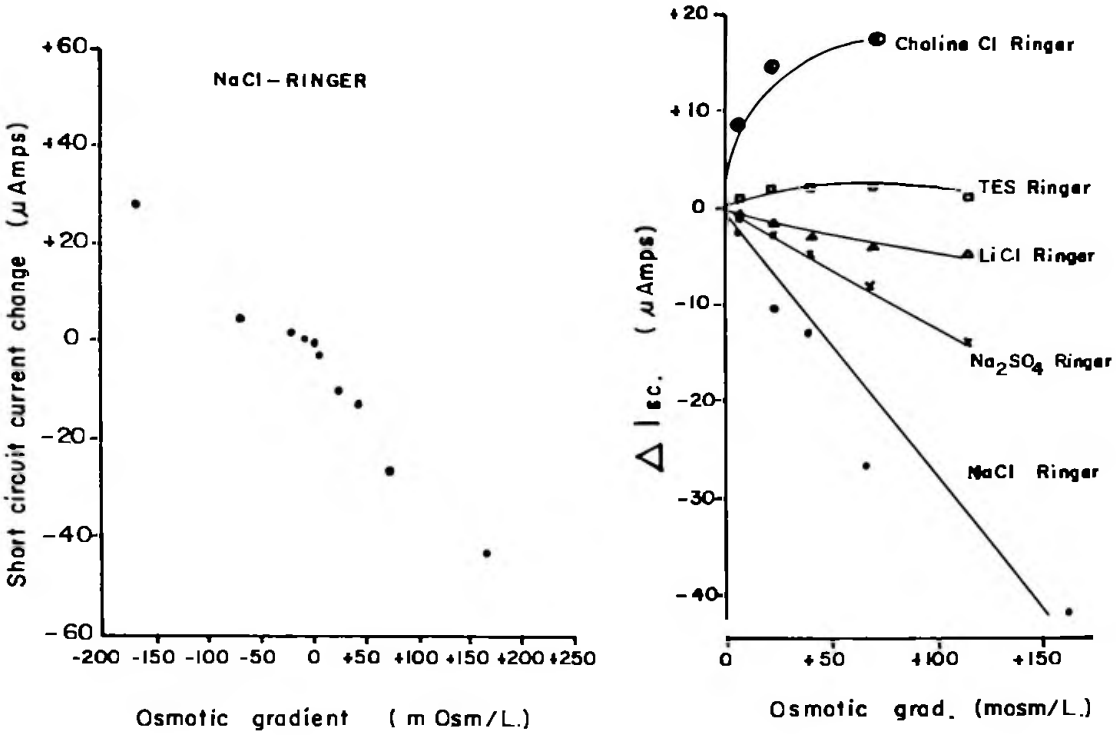

Figure 1. Effect of osmotic gradient on electrical measurement of flounder intestine.

and I_{sc} shifted toward positive values. The data suggest (1) that the intestinal membrane of flounder is more permeable to cations (Na or Li) than to anions (Cl); (2) unidirectional cation flux may be influenced by bulk flow of gluid moving in response to an imposed difference in osmotic pressure across the membrane.

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1970 #20

COMPENSATORY GROWTH OF THE INTESTINAL MUCOSA DURING SEAWATER ADAPTATION IN THE EEL (Anguilla rostrata): A BIOCHEMICAL ANALYSIS

Ralph Janicki and William Mackay, Allegheny General Hospital, Pittsburgh, Pa., and the Department of Biology, Case Western Reserve University, Cleveland, Ohio

The migration of euryhaline fish to seawater involves a number of complex physiological adaptations. For example, after 3 weeks of seawater adaptation in the eel (Anguilla rostrata), intestinal H_2O absorption is markedly increased (Bull. MDIBL 9:16-18, 1969). In the intestinal mucosa, oxygen consumption (Bull. MDIBL 9:23-26, 1969), and the activity of Na^+ - K^+ ATPase (Bull, MDIBL 8:32-34, 1968; see also Mackay and Janicki this volume) are twice that seen in freshwater eels. In this report, some of the cellular and biochemical events which accompany

intensified physiological activity in the intestines of the euryhaline eel during seawater adaptation are described.

Eels were maintained in tanks with running freshwater. For seawater adaptation, eels were kept for 2 days in 50% seawater before placing them in full strength seawater, where they were maintained for 3 weeks.

The entire intestine, from the pyloric sphincter to the anus was excised. The intestines were cut longitudinally and the excess water was blotted away. The mucosa was scraped from the serosal surface with a razor blade. Homogenates were prepared in ice-cold distilled H_2O, and the protein of this suspension was determined by the biuret method. After a series of perchloric acid and KOH extractions, colorimetric determinations of RNA and DNA were made using orcinol and diphenylamine respectively.

The mucosal weight (mg mucosa/kg body weight) in 12 freshwater eels was 2.69 ± 0.15, whereas in 12 seawater eels it was 3.55 ± 0.11 (+32%; $P < 0.001$). On the other hand, the serosal tissue was unaffected by adaptation to a higher salinity. The serosal weight (mg serosa/kg body weight) in 12 freshwater eels was 9.33 ± 1.07, whereas in 12 seawater eels it was 9.89 ± 0.97).

It seemed of interest to analyze more closely the biochemical composition of the intestinal mucosa of freshwater and seawater adapted eels. These data are presented in Table 1.

Table 1

PROTEIN, RNA AND DNA IN THE INTESTINAL MUCOSA OF SEAWATER AND FRESHWATER ADAPTED EELS

Treatment	mg protein / gram	mg RNA / gram	mg DNA / gram	mg protein / mg DNA	mg RNA / mg DNA
Seawater	133 ± 3*	7.82 ± 0.84	9.63 ± 0.76	14.42 ± 0.86	0.86 ± 0.13
Freshwater	129 ± 5	6.66 ± 0.34	9.20 ± 0.49	14.36 ± 0.86	0.74 ± 0.05
% Change	+ 3%	+ 17%	+ 5%	None	+ 16%
p Value	N.S.†	N.S.	N.S.	N.S.	N.S.

*All values are the Mean ± SE, with 10 eels in each group.

†Denotes not significant, $P > 0.05$.

Seawater adaptation failed to alter significantly the concentrations (mg/gram) of protein, RNA or DNA in the intestinal mucosa. Protein/DNA and RNA/DNA ratios were similarly not significantly altered.

Thus, seawater adaptation in the euryhaline eel leads to a significant accretion of intestinal mucosa. On a cellular level, two mechanisms could be involved in this growth response: hyperplasia (cell division), and hypertrophy (cell enlargement). Biochemical analyses fail to show significant changes in DNA concentration, or the protein/DNA and RNA/DNA ratios, which would be expected if cell enlargement were the mechanism of growth. We conclude therefore, that the increase in mucosal weight is due to an increase in cell number.

An increased number of mucosal cells would arise by increasing the mitotic activity in the intestinal crypts, or by decreasing the rate at which cells are sloughed into the lumen. Future

studies involving the use of labeled precursors are planned to elucidate the mechanism involve

This is the initial report describing the cellular and biochemical changes which occur in th intestines of a euryhaline fish during seawater adaptation. In part, euryhaline fish overcome the desiccative effects of hypertonic seawater by drinking, and increasing the rate of monovalent ion transport across the intestines. We speculate that successful adaptation is dependent upon a compensatory growth response to this intensified physiological activity in the intestine.

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1970 #21

DDT: ITS EFFECT ON INTESTINAL WATER ABSORPTION AND Na-K-ATPase IN TELEOSTS

Ralph Janicki, Jo Lingis, and William Kinter, Allegheny General Hospital, Pittsburgh, Pa., and the Upstate Medical Center, Syracuse, N. Y.

Teleosts are extremely sensitive to organochlorine pesticide poisoning (reviewed by Johnson, Trans. Am. Fish Co. 77:398-424, 1968). Unfortunately, pesticide concentrations in the marine environment continually increase due to agricultural run-off, a problem compounded by low pesticide biodegradability. In an attempt to elucidate the causal factors involved in organochlorine sensitivity in teleosts, the effect of DDT on osmoregulation is being investigated.

Marine and marine adapted euryhaline teleosts have overcome the problem of desiccation by their hypertonic environment. Tissue hypotonicity is maintained by drinking seawater and actively transporting salt, and with it water, across the intestinal epithelium Salts are eventua excreted by the gills; and water is retained.

The effect of DDT on intestinal salt and water absorption was measured in isolated gut sac of seawater adapted (2-3 weeks) eels (Anguilla rostrata), using a technique previously described by MacKay (Bull. MDIBL 9:23-26, 1969). DDT (10 mg/ml) was dissolved in N,N-dimeth formamide (DMF). The isotonic saline solution contained 50 parts per million (ppm) DDT, and 0.5% DMF. In these preparations, similar concentrations of dimethyl sulfoxide and ethanol had strong inhibitory effects on water absorption. All intestinal preparations were preincubated in their respective solutions for 1 hr, with repeated flushing, at 2-5°C. Eel guts were then incuba ed for 1 hr at 15°C; and water absorption was calculated as μl H_2O/g intestine x hr. Values ar the mean ± SE.

In 7 guts incubated in isotonic saline, H_2O absorption was 281 ± 30. In 7 guts incubated in isotonic saline containing 0.5% DMF, H_2O absorption was 250 ± 24, not significantly different from isotonic saline along ($P > 0.3$). However, 7 guts incubated in a fine suspension of 50 ppm DDT (1.4×10^{-4}M) in isotonic saline containing 0.5% DMF, H_2O absorption was reduced to 133 ± 26 (-47%; $P < 0.01$).

Water absorption is believed to passively follow active ionic transport. We therefore investigated the effect of DDT on intestinal mucosal Na-K-ATPase activity, the enzyme believed to be responsible for the transport of sodium across cell membranes. For a review see Skou (Physiol. Rev. 45:596-617, 1965).

Na-K-ATPase activity was measured on whole homogenates of intestinal mucosa using a