inhibition of Na^+, K^+, Mg^{2+}-ATPase activity by dimethyl sulfoxide was unexpected since it is generally considered to be an innocent vehicle for the administration of insoluble compounds. Also noteworthy was the complete inhibition of all ATPase activity by cyclohexanone, an often used commercial solvent for DDT.

An investigation of the effects of DDT, DDE, and DDD on Na^+, K^+, Mg^{2+}-ATPase activity in *S. acanthias* rectal gland followed. At a final concentration of 50 parts per million (ppm) none of these organochlorines significantly inhibited Na^+, K^+, Mg^{2+}-ATPase. In view of our previous studies with teleosts, which showed DDT inhibits Na^+, K^+, Mg^{2+}-ATPase from the intestinal mucosa and gill epithelium in a variety of marine and marine adapted euryhaline teleosts, our results with enzyme from the rectal gland of *S. acanthias* were unexpected. Accordingly, at least in terms of DDT sensitivity, Na^+, K^+, Mg^{2+}-ATPase from the gill and intestine of teleosts appears to differ significantly from that found in *S. acanthias* rectal gland.

On the other hand, Mg^{2+}-ATPase in *S. acanthias* rectal gland was inhibited by DDT, DDE and DDD. It is of interest that DDE, which is not insecticidal, had the least effect on Mg^{2+}-ATPase (Figure 1). The flattening of the curves suggests the presence of more than one Mg^{2+}-ATPase, and this can in part be attributed to the use of whole homogenates. A study of the degrees of sensitivity of different Mg^{2+}-ATPases to DDT is in progress. The inhibition of Mg^{2+}-ATPase by less than 10 ppm DDT, DDE, and DDD in *S. acanthias* rectal gland may be of critical importance. In contrast to the exiguous concentrations of DDT and DDE found in autochthonous invertebrates and teleosts in Frenchman Bay, *S. acanthias* liver, on a wet weight basis, is already contaiminated with approximately 3 ppm DDT and 1 ppm DDE (Bull. MDIBL 9:2-4, 1969). And although it is hazardous to extrapolate directly from the *in vitro* to the *in vivo* situation, the possibility exists that present levels of contamination are already affecting *S. acanthias.* (Supported by Grant AMO6479, from the U.S.P.H.S.)

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DDT AND THE DISRUPTION OF OSMOTIC REGULATION IN SEA-WATER ADAPTED EELS *(Anguilla rostrata).*

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In part, marine teleosts overcome the desiccative effects of sea water by drinking and absorbing water, along with Na^+, across the intestinal epithelium. Sodium ions are eventually secreted by the gill while water is retained. The insecticide DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] disrupts water absorption across the intestinal epithelium. This functional disruption has an enzymatic explanation, since DDT inhibits intestinal mucosal Na^+, K^+, Mg^{2+}-ATPase, and in some cases Mg^{2+}-ATPase (Science 173:1146-1148, 1971; Nature 233:148-149, 1971). These results raised the possibility that the toxicity of DDT in teleosts may involve the disruption of osmoregulation, and this study tends to substantiate that view.

The effect of DDT on osmoregulation was determined in eels which had been maintained in sea water for 3 weeks. Eels, weighing between 87 and 141 grams, were placed in aluminum trays containing 2 liters of sea water which was either 0.1% ethanol (controls), or 0.1% ethanol and 1 part per million (ppm) DDT (experimentals), at 15° C. After 3 hr, DDT treated eels showed a significant increase in Plasma $[K^+]$, but no increase in osmolality or Na^+ (Table 1). After 6 hr of DDT exposure,

Table I

Effect of 1 ppm p,p'-DDT on Plasma Osmolarity, Sodium and Potassium in Sea-Water Adapted Eels (Anguilla rostrata).

Treatment	N	Osmolarity	Na^+ mEq/L	K^+ mEq/L
Control	5	358 ± 10 †	163 ± 2	2.40 ± 0.25
p,p'-DDT - 3 hr.	6	349 ± 8	161 ± 2	4.64 ± 0.39 *
p,p'-DDT - 6 hr.	5	462 ± 10 *	192 ± 3 *	5.38 ± 0.13 *

† Values are the mean ± SE; control values were collected at 3 and 6 hr.

* Significantly different from control ($P<0.01$).

there were significant increases in plasma osmolality, Na^+, and K^+ concentrations. Pronounced hemolysis, an increase in respiratory activity, and convulsions accompanied the changes in plasma chemistry. These results suggest that one of the symptoms of DDT toxicity in teleosts is the disruption of osmoregulation.

In order to determine the effect of DDT on active Na^+ transport in eel gill, we devised an *in vitro* method for assessing the ability of gill epithelium to maintain a Na^+ gradient against sea water. Gill arches from marine adapted eels were placed in artificial sea water (Annot. Zool. Japon. 40:123-129, 1967) containing varying amounts of ^{22}Na. After incubating the oxygenated gills at 15° C for 1 hr, with shaking, the filaments were stripped from the gill arches, weighed and counted, as were the respective media. The results are expressed as the mean ± SE of the filament to media ratio, with 15 gills in each group. When eel gills were incubated in straight sea water, the ratio was 0.114 ± 0.01. The solvent N,N-dimethylformamide (DMF), at a final concentration of 0.5%, had no measurable effect on this assay; the ratio was 0.112 ± 0.00. However, when the artificial sea water contained 50 ppm DDT suspended in 0.5% DMF, the ratio was increased to 0.162 ± 0.01 ($P<0.01$). Thus, the ability of eel gill filaments to maintain a Na^+ gradient against sea water was impaired by DDT. Since similar preparations are sensitive to 2,4-dinitrophenol and ouabain, active transport processes appeared to be involved.

Na^+,K^+,Mg^{2+}-ATPase is generally considered to be an intrinsic factor in the active transport of Na^+. We therefore determined the effects of 50 ppm DDT on this enzyme in 600 x g supernatant fractions of eel gill filaments homogenized in 0.25 M sucrose, 0.005 M EDTA and 0.03 M imidazole (adjusted to pH 6.8). The assay was conducted using a previously described procedure (see Nature and Science references). Expressed as μmoles Pi/mg protein x hr at 15° C, Na^+,K^+,Mg^{2+}-ATPase activity in 5 duplicate assays containing 5% DMF for solvent was 0.37 ± 0.07, whereas in 5 duplicate

assays containing 5% DMF and 50 ppm DDT, it was 0.23 + 0.03 (-38%; PL0.01). Accordingly the disruptive of Na^+ transport by DDT in intact gills may be related to Na^+,K^+,Mg^{2+}-ATPase inhibition.

Summarizing these data and our previous reports, marine adapted eels lose the capacity to maintain plasma osmolarity after being exposed to DDT. The primary osmoregulatory organs in marine teleosts are the intestine and the gill. DDT disrupts sodium dependent water absorption across isolated intestines, as well as the ability of gill filaments to maintain a Na^+ gradient against sea water. The disruption of sodium transport appears to have an enzymatic basis since DDT inhibits Na^+,K^+,Mg^{2+}-ATPase in the intestinal mucosa and gill filaments.

Finally, the osmotic gradient between the body fluids of teleosts and the bathing medium is greater in sea water than in fresh water. It can be reasoned, therefore, that teleosts should be more sensitive to DDT in sea water than in fresh water. To test this we studied the percent mortality versus time of marine adapted killifish *(Fundulus heteroclitis)*in sea water containing 0.1% ethanol and 1 ppm DDT; and, fresh water containing 0.1% ethanol and 1 ppm DDT. Killifish were maintained in aluminum trays at 15° C. There were 20 killifish in each group, each fish representing 5% of the data. Mortality was linear with time in both graphs. However, whereas 50% of the killifish in sea water died within 12 hr, the time at which 50% of the fresh water killifish died was delayed to 24 hr. (There was no mortality in control fish, trays containing sea water and fresh water with 0.1% ethanol.) DDT appears therefore to be more toxic to teleosts in sea water than in fresh water environments. Moreover, the increased sensitivity in sea water is consistent with the hypothesis that osmoregulatory disruption is involved in the toxicity of DDT in teleosts. (Supported by Grant AM06479, from the U.S.P.H.S.)

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DISTRIBUTION OF BLOOD FLOW IN *S. Acanthias:* A PRELIMINARY STUDY

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The distribution of blood flow through the gills and the parallel elements of the arterial circulation were determined in the intact circulation of the dogfish.

Eleven free-swimming and four partially restrained male dogfish (1.49 $\pm$ 0.61 Kg) were used. In the first group an injection catheter was threaded into either the dorsal aorta to the level of the fifth gill arch or into the caudal artery 3 cm caudal to the cloaca through a #15 Touhuy needle inserted into the caudal artery. The fish swam freely in an 0.3 cubic meter seawater tank. In the restrained group the heart was exposed through a midline incision and the injection catheter was inserted into the ventricle through the apex. The same protocol was used for both groups of animals. Blood samples were taken from the injection catheter at time 0 (time when the cannula was approximately positioned), 30 minutes, and one and one-half hours later, for oxygen saturation, pH, equilibrated pH (abstract #27 MDIBL 1967), and hematocrit. At 30 minutes, 1cc of 15μ diameter ^{141}Ce tagged and 1cc of 50 μ diameter ^{125}I tagged microspheres (3M Brand Tracer Microspheres) were given and washed in by 1cc of dogfish Ringers. The amount of activity in each dose was