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DDT, DDE, AND DDD: EFFECTS ON ATPase ACTIVITY IN THE RECTAL GLAND OF THE DOGFISH SHARK *(Squalus acanthias).*

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In an effort to elucidate the toxicity of the insecticide DDT and related compounds in marine animals, we have been investigating the inhibition of ATPase activities, and the disruption of sodium transport in osmoregulatory tissues of certain teleosts (Nature 233:148-149, 1972; Science 173: 1146-1148, 1971; also Janicki and Kinter, Bull. MDIBL, this volume). It seemed of interest to extend our studies to the elasmobranchs, and explore the effects of DDT and its analogues on ATPase activities in the rectal gland. The rectal gland secretes excess sodium against a concentration gradient, and has high Na^+, K^+, Mg^{2+}-ATPase activity.

Dogfish *(Squalus acanthias)* were killed and the rectal glands excised and kept frozen at -70° C until used. Methods of preparing whole homogenates, conducting Mg^{2+}-ATPase and Na^+, K^+, Mg^{2+}-ATPase assays at 15° C, and the colorimetric determination of phosphate were previously described in detail (see Nature and Science references, above).

TABLE 1

Effect of Organic Solvents (5% Final Concentration) on ATPase Activity in the Rectal Gland of the Dogfish (Squalus acanthias).

	% Inhibition ATPase*	
Organic Solvent	Mg^{2+}	Na^+, K^+, Mg^{2+}
N, N-dimethylformamide	37	+12†
acetone	49	9
ethanol	60	33
dimethyl sulfoxide	29	60
cyclohexanone	98	95

* Values are the average of 3 experiments

† N, N-dimethylformamide mildly stimulates Na^+, K^+, Mg^{2+}-ATPase in S. acanthias rectal gland.

The organochlorines: p,p'-DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane]; p,p'-DDE [1,1-dichloro-2, 2-bis (p-chlorophenyl) ethylene]; and, p,p'-DDD [1,1-dichloro-2, 2-bis (p-chlorophenyl) ethane] are extremely insoluble in aqueous media. These organochlorines can, however, be kept in solution or light suspension in an enzyme assay by using an organic solvent. Accordingly, we determined the inhibitory effects of several organic solvents (at a final concentration of 5%) on Mg^{2+}-ATPase and Na^+, K^+, Mg^{2+}-ATPase activities in the rectal gland of *S. acanthias* (Table 1). N,N-dimethylformamide (DMF) proved to be the most desirable organic solvent tested. The marked

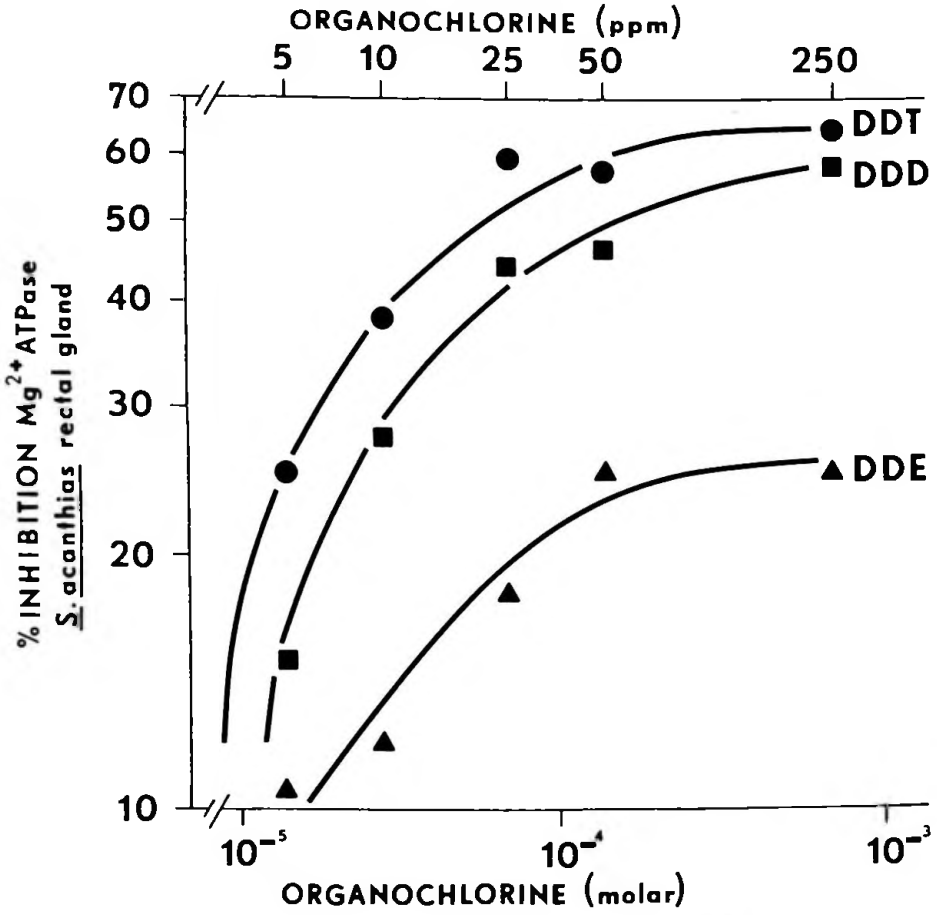

Figure 1. A dose response curve depicts the inhibitory effects of DDT, DDE and DDD on Mg^{2+}-ATPase activity from *S. acanthias* rectal gland. DDT, DDE and DDD concentrations range between 5 and 250 ppm (top axis), which is equal to between 1.4×10^{-5} and 7.0×10^{-4} molar for DDT (bottom axis). The linear relationship which would be expected in a single enzyme system by plotting the log-percent Mg^{2+}-ATPase inhibition against the log concentration of DDT, DDE and DDD was not found. Major portions of Mg^{2+}-ATPase activity are sensitive to DDT and DDD, and a smaller portion to DDE. The remaining Mg^{2+}-ATPase activity is apparently resistant to these organochlorines.

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,