these means differ from the grand mean of 76.78 by more than 0.5 beat per minute.

5. Separate averaging as a function of the counterbalancing procedure indicated that the general trends of Fig. 1 were equally manifest regardless of whether the subjects began the experiment by using the covert (that is, without prior motor experience) or overt response mode. The unexpected finding of equally large cardiac deceleration in the slow (versus fast) conditions may possibly reflect the high degree of attention (to the details of responding) required by the multiresponse nature of the task.
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8 March 1971; revised 7 June 1971

DDT: Disrupted Osmoregulatory Events in the Intestine of the Eel Anguilla rostrata Adapted to Seawater

Abstract. *The drinking of seawater and absorption of water along with sodium across the intestinal epithelium are well-known osmoregulatory events in marine teleosts. The insecticide DDT impairs fluid absorption in intestinal sacs from eels adapted to seawater. Furthermore, this functional impairment has an enzymatic basis; DDT also inhibits the (Na^+ and K^+) activated, Mg^{2+}-dependent adenosine triphosphatase in homogenates of the intestinal mucosa. Thus, the extreme sensitivity of teleosts to organochlorine pollutants may involve the disruption of osmoregulatory transport mechanisms.*

In view of the importance of marine fisheries to human nutrition (*1*), it is particularly alarming that fish are the most sensitive of all vertebrates to widespread organochlorine pollutants such as DDT (*2*). Although it is generally accepted that in both vertebrates and invertebrates DDT exerts a direct toxic effect on the nervous system, the biochemical basis is still uncertain (*3*). Because some evidence exists for inhibition of (Na^+ and K^+) activated transport adenosine triphosphatase in several organs including brain (*4*), we wondered if the extreme sensitivity of fish to DDT might not reflect parallel disruptions of osmoregulation and nerve function.

Marine teleosts face desiccation in their hypertonic environment. In part, they preserve tissue hypotonicity by drinking seawater and absorbing sodium and chloride across the intestinal epithelium. Water follows the absorption of these ions and is retained in the body while the ions are secreted by the gill epithelium (*5*). The adenosine triphosphatases appear to function in these osmoregulatory processes. The (Na^+ and K^+) activated, Mg^{2+}-dependent adenosine triphosphatase [(Na^+,K^+,Mg^{2+}) adenosine triphosphatase] which is sensitive to ouabain is believed to be involved in the transport of sodium across cell membranes (*6*). Supporting this hypothesis is the fact that the activity of this enzyme in the intestinal mucosa of eels adapted to seawater is twice that seen in eels adapted to freshwater (*7*). Furthermore, there is evidence that the mitochondrial portion of the (Mg^{2+}) adenosine triphosphatase which is the portion stimulated by 2,4-dinitrophenol is involved in oxidative phosphorylation (*8*). Thus by supplying ATP, this enzyme may be at least indirectly involved in active transport.

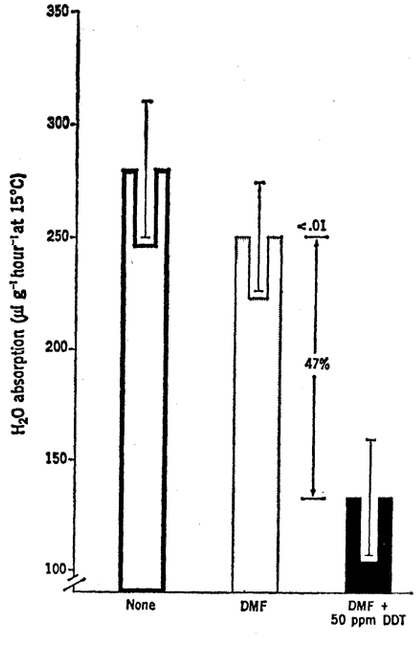

Fig. 1. The effect of DDT (final concentration 50 ppm, or 1.4×10^{-4} mole/liter) on water absorption in the intestine of the eel. The addition of 0.5 percent *N,N*-dimethylformamide (DMF) to the Ringer solution has no significant effect on intestinal water absorption ($P > .3$). The addition of 50 ppm of DDT in DMF decreases water absorption 47 percent ($P < .01$). The tops of the bars represent means; the vertical lines represent standard errors. Seven intestinal sacs were assayed in each medium.

Eels (*Anguilla rostrata*) between 30 and 38 cm long were captured in estuaries along the Maine coast. They were adapted to and maintained in seawater (13° to 16°C) for 3 weeks before use. After decapitation, the intestines from the pyloric sphincter to the anus were excised. Intestines were cannulated and prepared for measurement of water absorption by means of a procedure in which noneverted sacs are used (*9*). Analytical grade *p,p'*-DDT (*10*) was dissolved in *N,N*-dimethylformamide (DMF) at a concentration of 10 mg/ml. Three media were used: Ringer, Ringer containing 0.5 percent DMF, and Ringer containing 0.5 percent DMF and a suspension of 50 parts of DDT per million (ppm). Each cannulated intestine was first incubated in its respective medium for 60 minutes at 2° to 5°C; the medium was repeatedly rinsed through the lumen to allow DDT to enter the tissue. Then, each intestine was filled with its respective medium under slight hydrostatic pressure and sealed. The sacs were then incubated at 15°C in erlenmeyer flasks gassed with oxygen and containing additional medium. Thus, when present, DDT was on both the serosal and mucosal sides. Water absorption was calculated as sac weight at time 0 minus weight at 60 minutes and presented as microliters of H_2O per gram of intestine per hour.

When isolated sacs of eel intestine were incubated in $1.4 \times 10^{-4}M$ DDT (50 ppm), there was a 47 percent

($P < .01$) inhibition of water absorption (Fig. 1). This is a strong inhibition and is comparable to the effects of more familiar blocking agents. For example, $1 \times 10^{-4}M$ 2,4-dinitrophenol and $1 \times 10^{-4}M$ ouabain also inhibit approximately 50 percent of the intestinal water absorption in eels adapted to seawater (*11*). However, DDT and, to a lesser extent, 2,4-dinitrophenol are lipophilic, and since membranes contain lipids, the concentrations of these compounds at specific transport loci may have been greater.

We also correlated the impairment of water absorption with the inhibition of activities of adenosine triphosphatase in homogenates of intestinal mucosa. For each determination of (Na^+, K^+,Mg^{2+}) and (Mg^{2+}) adenosine triphosphatases the intestinal mucosae of six eels were pooled and homogenized (2 percent, weight per volume) in a mixture of 0.25*M* sucrose, 0.005*M* disodium ethylenediaminetetraacetic acid, and 0.03*M* imidazole (adjusted to *p*H 7.4). Deoxycholate was omitted because it partially inhibited (Na^+, K^+,Mg^{2+}) adenosine triphosphatase. The assay medium (*7*) contained in final concentration 20 m*M* imidazole (*p*H 7.8) and either 100 m*M* NaCl and 20 m*M* KCl, or 120 m*M* NaCl. For the enzyme studies, DDT was dissolved in DMF. At a final concentration of 5 percent, DMF had no effect on (Na^+,K^+,Mg^{2+}) adenosine triphosphatase and inhibited only 20 percent of (Mg^{2+}) adenosine triphosphatase activity. Before addition of ATP, the tubes were incubated for 30 minutes at 15°C (*12*). Reactions were initiated by the addition of 0.3 ml of a solution containing in final concentration 100 m*M* $MgCl_2$ and 100 m*M* disodium salt of adenosine triphosphate (neutralized with saturated tris buffer). The final volume was 5.0 ml. After 30 minutes at 15°C, the reactions were terminated by addition of 1.0 ml of ice-cold 30 percent trichloroacetic acid; the tubes were placed in ice for 10 minutes. The precipitate was removed by centrifugation. Because DDT interfered with the colorimetric procedure for determination of phosphate (*13*), it was extracted from a 3.0-ml portion of the supernatant with an equal volume of ice-cold toluene.

The DDT had a strong inhibitory effect on (Na^+,K^+,Mg^{2+}) adenosine triphosphatase (Fig. 2). Even at the low concentration of 5 ppm of DDT (1.4×10^{-5} mole/liter), there was 43 percent inhibition. The concentration of DDT which inhibited 50 percent of the activity was approximately 15 ppm (4.0×10^{-5} mole/liter). We were unable to determine whether (Na^+,K^+, Mg^{2+}) adenosine triphosphatase can be completely inhibited by DDT. Suspensions of 250 ppm with 5 percent DMF were already very cloudy; thus it seemed unreasonable to go to higher concentrations. The (Mg^{2+}) adenosine triphosphatase in intestinal homogenates of the eel was also inhibited by DDT, albeit to a lesser extent (Fig. 2). The break in the dose response curve suggests the presence of two (Mg^{2+}) adenosine triphosphatases—one smaller component which is sensitive to DDT and a larger fraction which is resistant. The question arises as to whether previous environmental exposure to DDT was, to some degree, already suppressing adenosine triphosphatase activity in the eels used in the experiment. Although we do not know the extent of previous contamination, whole-body concentrations of DDT in other teleosts inhabiting the Frenchman Bay area have been reported as 0.033 ppm or lower (*14*), an amount which presumably would have had little effect on our data.

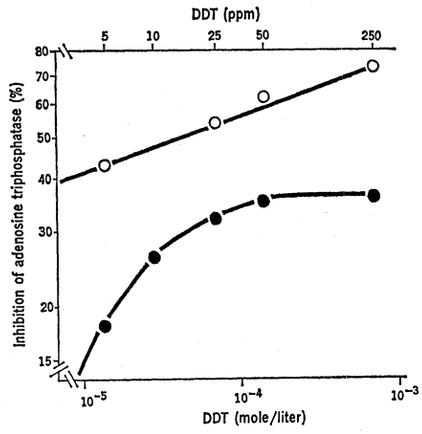

Fig. 2. The relationship between the concentration of DDT and the percentage of inhibition of (Na^+,K^+,Mg^{2+}) and (Mg^{2+}) adenosine triphosphatases in the intestinal mucosa of the eel. The log percentage of the inhibition is plotted against the log concentration of DDT in molarity and parts per million. This procedure establishes a linear relationship between activity and the concentration of the inhibitor (*20*).

Intestinal water absorption in eels adapted to seawater is inhibited in vitro by cyanide, 2,4-dinitrophenol, and ouabain (*11*), and represents, in all likelihood, a coupled process involving sodium, the major cation transported across the intestine (*15*). Our data demonstrate that DDT impairs water absorption apparently by inhibiting mucosal adenosine triphosphatases involved in sodium transport. A reasonable correlation between enzyme activity and function exists, since at 15°C the normal temperature for these eels, the activity of (Na^+,K^+,Mg^{2+}) adenosine triphosphatase and water absorption are each about halved by 15 and 50 ppm of DDT, respectively. Although adenosine triphosphatases and sodium secretion in the gills may also prove sensitive, our findings have significance in that many species of teleosts are reported to die at whole-body concentrations commonly averaging between 5 and 10 ppm of DDT (*16*). Accordingly, the extreme sensitivity of fish to DDT may involve inhibition of adenosine triphosphatases and an accompanying disruption in osmoregulation.

The inhibition of adenosine triphosphatase activity in teleosts seems to be a general property of organochlorine insecticides. In preliminary surveys (*17*) DDT inhibited (Na^+,K^+,Mg^{2+}) adenosine triphosphatase, but not (Mg^{2+}) adenosine triphosphatase in the intestinal mucosae of several other marine species, and both of these enzymes in the gill of winter flounder (*Pseudopleuronectes americanus*). In the lake trout, the organochlorine insecticides chlordane, dicofol, lindane, and DDT inhibit (Mg^{2+}) adenosine triphosphatase in brain, liver, and muscle, and (Na^+,K^+,Mg^{2+}) adenosine triphosphatase in brain (*18*). Furthermore, disrupted osmoregulation, evidenced by an alteration in the concentration of serum electrolytes, has been reported in both northern puffers and goldfish treated with the organochlorine insecticide endrin (*19*). These observations tend to substantiate our hypothesis that the sensitivity of teleosts to organochlorine insecticides involves impairment of osmotic regulation.

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5 April 1971

Retention Deficit Correlated with a Deficit in the Corticoid Response to Stress

Abstract. *Previous research has indicated that intracerebral injection of potassium chloride, and the resultant electrical silence of the brain, lead to the attenuation of a previously conditioned emotional response; this response may reflect conditioned fear. The data reported here indicate that the normal mobilization of corticosteroids, perhaps a second index of emotionality, is also attenuated by such injections.*

Avis and Carlton and later Hughes (*1*) demonstrated that temporary depression of the hippocampal electrical activity can produce a deficit in the retention of a previously conditioned emotional response (CER). The CER refers to the fact that an animal will suppress its ongoing behavior when a conditioned stimulus, previously paired with shock, is presented. Animals that have received intrahippocampal injections of KCl, which produce electrical silence of brain activity at the site of injection, do not show such suppression.

One question that these data pose is whether the deficit is a reflection of a general deficit in the ability to inhibit behavior or whether this behavioral deficit reflects a deficit in fear. If the latter were true, one would expect animals that had been given KCl to show a deficit in some independent measure of fear. Such a deficit would at least suggest that the relative absence of conditioned suppression was due to an attenuation of fear rather than to a general inability to inhibit ongoing behavior.

In an initial attempt to resolve this question, Avis (*2*) measured the cardiac deceleration, perhaps a measure of conditioned fear, that accompanies presentation of the conditioning stimulus. He found that animals that have undergone electrical depression do not display such cardiac deceleration.

A second measure that may reflect emotionality is examined in the experiment reported here. The results point in the same direction: Intrahippocampal injections of KCl, and consequent electrical silence, attenuate the usual elevation in corticosteroids that accompanies presentation of a conditioned fear stimulus (*3*).

Nineteen male albino rats (Sprague-Dawley strain) were the subjects (*4*). Briefly, all animals were trained to lick from a water-filled tube and, several days later, were given four shocks, each preceded by a tone presentation. After intervening hippocampal injections (see below), the subjects were tested for suppression of drinking in the presentation of the tone. The degree of drinking suppression was taken as a measure of conditioned fear. Immediately after this test session, the subjects were decapitated and blood was collected. The adrenals of each rat were also removed, weighed, and homogenized. The serum and adrenal samples were analyzed for corticosterone.

Table 1. Mean values for animals given KCl and saline.

Suppression	Plasma (μg/100 ml)	Adrenal (μg/100 g)
	*Potassium chloride**	
25.90	18.37	3.09
(10.68)†	(4.21)	(0.89)
	Saline‡	
259.90	40.04	6.96
(20.81)	(7.19)	(0.96)

* Nine animals. † Standard error of the mean. ‡ Ten animals.

The details of the procedure are as follows. On day 1, animals deprived of water for 24 hours were placed in a response chamber with a water-filled drinking tube in one corner. All animals were allowed to make 110 licks. Three days later each animal was returned to the chamber, the drinking tube having been removed, and was given four tone-shock pairings, each separated by 1 minute. Each 15-second tone presentation was terminated by a 1-second, inescapable, 2-ma shock delivered to the animal's feet through the grid floor of the chamber. Twenty-four hours after the tone-shock pairings, all animals were anesthetized with pentobarbital (40 mg per kilogram of body weight) and chloral hydrate (165 mg/kg). Either KCl or saline was then bilaterally injected into the hippocampus. Two stainless steel, 23-gauge hypodermic tubes, insulated to within 1 mm of the tip and mounted on a Plexiglas holder, were used for electrical recording and injecting. With the aid of a stereotaxic instrument, these tubes were lowered into place. The placements were as follows: 5 mm posterior to bregma, 5 mm lateral to the midline suture, and 5 mm below the dorsal surface of the cortex (*5*). Monopolar recordings were made with the skin retractors serving as the indifferent electrode. Electroencephalographic (EEG) recordings were made with a Beckman type RB dynograph. For further procedural details see Avis and Carlton as well as Hughes (*1*).

The EEG recording began as soon as the electrodes were lowered into place. Once the EEG activity stabilized (5 to 10 minutes), a 30-gauge tube connected to a Gilmont microinjector was inserted through the electrodes. A 25 percent solution of KCl was injected into each side of the hippocampus. At least 11 minutes of electrical silence (little or no wave amplitude at an amplifier gain of 200 μv) was induced in all cases. From 3 to 7.5 μl of KCl were needed to produce this degree of electrical depression; comparable volumes of physio-